A Study of the Chromosomes of the Germ Cells of Metazoa.

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A STUDY OF THE CHROMOSOMES OF THE GERM CELLS OF METAZOA.

Plates IV—VIII.

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I. Introduction.

The present study is practically a continuation of previous work of mine upon spermatogenesis in the Arthropods. It was undertaken primarily to correct certain errors of interpretation and observation in my work on *Pentatoma* (*Euchistus*). But many morphological problems arose in connection with this reëxamination, such as the significance of the changes in the synapsis stage, the significance of the chromatin nucleoli, the reasons for a reduction division, the significance of the sequence of the stages of the germinal cycle, and the question as to why different species have different numbers of chromosomes. Thus my investigations given here are essentially on the history of the chromosomes during the germinal cycle.

It is impossible to answer these problems by an examination of a single species, and accordingly there are presented here the results of a comparative study of the spermatogenesis of some forty-two species of *Hemiptera heteroptera*, belonging to twelve different families. This comparative study has brought to light certain wholly unexpected phenomena, and none less anticipated than the discovery of four species with an uneven normal number of chromosomes; this discovery has furnished facts for explaining how the chromosomal numbers may change with the evolution of the species, and how the chromatin nucleoli may have originated. And only such a comparative study could furnish facts to show that in the synapsis stage bivalent chromosomes are formed by the union of paternal with maternal chromosomes—*i. e.*, that this is the stage of conjugation of the chromosomes. The comparative method in Cytology cannot be overestimated, though of course careful detailed examinations of single objects should be carried on at the same time. For a single object is rarely capable of serving as the basis of explana-

tion of all the problems; an investigation of a number of forms always shows that some are more favorable than others for answering certain questions, and then there is the chance that a wholly unexpected discovery may be made that may have great significance. So the plea is made here for the comparative method in Cytology, and Cytology should not be ranked as a line of work separate from others—it is all Morphology in the broad sense of the term, and it only happens that in Cytology we use higher magnification powers of the microscope than in other lines. If one form shows phenomena that seem inexplicable after careful work, then the proper method, the one that would promise a surer reaching of results, is not to reëxamine this form again and again, but to compare other forms in the search for the explanation.

In the present paper the part containing the general conclusions may appear disproportionally great to the record of the observations. These observations are to great extent on the number and valence of the chromosomes and chromatin nucleoli from the time of the last generation of the spermatogonia up to the formation of the spermatids. But the determination of these numbers is very difficult; large numbers of sections have to be examined in order to find the necessary stages, and the number of the chromosomes of each stage have to be counted in a considerable number of cells of each species in order to insure accuracy. The counting has been done in all cases by selecting those cells in which the chromosomes are most loosely grouped, being sure at the same time that all the chromosomes are in the plane of the section, drawing the chromosomes carefully with the camera lucida, then counting their number on the drawings. This demands much patience and time, necessitating also constant reëxaminations and study of new material, though the results may be tabulated in a very small space. Of course the difficulties are most pronounced where the chromosomes are numerous and small.

The material was collected by me at two localities—in the vicinity of Philadelphia, Pennsylvania, and in the neighborhood of Wood's Holl, Massachusetts. Great care was taken to insure accurate identification of the species, and my specimens were directly compared by me with the collections in the museums of the Wagner Institute of Science and of the Academy of Natural Sciences of this city; these collections had been labeled by Dr. P. R. Uhler, of Baltimore, our foremost American authority on this group of Insects; and I must also acknowledge my indebtedness to Dr. Uhler for kindly identifying a number of species which were not represented in the collections just mentioned. To my friend, Mr. C. W. Johnson, curator of the Wagner Institute, my thanks are also due for aid in identification. The differences of the spermatogenetic phenomena of different species shows how important it is to secure accurate identification.

The testes were removed as rapidly as possible from the living animals and immediately placed in the fixing fluids, Flemming's chromo-aceto-osmic acid mixture (the

stronger solution), Hermann's chromo-aceto-platinic chloride mixture, and a picro-acetic mixture recommended by Prof. Conklin (100 parts saturated aqueous solution of picric acid, 100 parts distilled water, 6 parts glacial acetic acid) being used. Of these the mixtures of Flemming and Hermann proved the best for the chromosomal structures, for the picro-acetic mixture, while giving an excellent preservation of the actromatic spindle structures, swells the chromosomes very considerably so that on pole views of monaster stages they generally appear closely apposed to one another, which makes it difficult to count them. Where the species is small it is necessary to remove the testes in the fixative under a dissecting microscope. The sections were stained either by the iron-hæmatoxylin method of Heidenhain or by the saffranine-gentian violet method of Hermann.

II. Observations.

PENTATOMIDÆ.

1. *Euchistus variolarius* Pal. Beauv.

This is the species the spermatogenesis of which I described under the name of "*Pentatoma*" in a former paper (1898); twenty-eight testes were studied from adult individuals of all seasons except the winter months.

In my former paper (*l. c.*) I did not find chromatin nucleoli in the spermatogonia; I concluded that there was no stage of longitudinal splitting of the chromosomes during the growth period, and I concluded that the second maturation division was a reduction mitosis like the first. Shortly afterward appeared the papers by Paulmier (1898, 1899) on the spermatogenesis of *Anasa tristis*, wherein he showed that there are two chromatin nucleoli (his "small chromosomes") in the spermatogonia, and that these unite in the spermatocytes to form one bivalent one; that the chromosomes undergo a longitudinal splitting in the growth period, and that the second maturation division is equational. In those points wherein I differed from Paulmier, I find that Paulmier is correct, and that I gave a wrong interpretation to the phenomena in *Euchistus*. I find nothing to correct in the matter of the other points described in my earlier account, and here give briefly merely the necessary emendations to my former paper.

Spermatogonia.—In the resting spermatogonium there are in the nucleus beside the true nucleolus (of which there may be more than one) two small chromatin nucleoli of rounded form (Plate I, Fig. 1, *N. 2*). With the saffranine-gentian violet stain of Hermann, when properly used, these stain bright red, the true nucleolus a faint bluish, the chromatin proper a deep violet; careful staining and thin sections are necessary to show them plainly. Sometimes one or both of them are attached to a true nucleolus. In the prophases of mitosis the chromatin nucleoli are easily recognizable by being much smaller

and more spherical than the chromosomes. In the monaster stage, in favorable cases where the chromatic elements are not too densely arranged, are seen fourteen larger elements, the chromosomes proper, and two smaller ones regularly rounded in form, which are the chromatin nucleoli (Figs. 2, 3, *N. 2*). Sometimes the chromosomes are rounded, but since they frequently appear slightly elongate on pole view of the spindle, their division in metakinesis must be an equational one. In the metakinesis all sixteen elements, the fourteen chromosomes and the two chromatin nucleoli, are divided, so that each daughter cell (first spermatocyte) receives sixteen elements.

Thus there are two chromatin nucleoli in the spermatogonia, and the chromatin nucleolus of the spermatocystes is not, as I had previously described, formed by a modification of one of the fourteen chromosomes of the spermatocytes, but is derived from the two of the spermatogonia. My error was perhaps excusable, since in restudying the preparations which were used for my former paper I find that they are not suitably stained to show the chromatin nucleoli in the spermatogonia.

Growth period of the spermatocytes (anaphases of the last spermatogonic division, synapsis, postsynapsis, telophase and rest).—The fourteen chromosomes in each daughter cell (first spermatocyte) pass toward the pole of the spindle and become irregular in contour and form. Then each becomes longitudinally split (Figs. 4–11). This splitting cannot be clearly seen in all preparations, and is by no means as clear as in *Anasa* and certain other *Hemiptera;* the preparations of my former paper were too deeply stained to show it. The split commences in the early synapsis stage (Fig. 4) and is most marked in the postsynapsis (Fig. 9), and is clearly a single longitudinal split. Never do the split halves separate widely from one another, as Paulmier found for *Anasa*, but always appear to remain close together and approximately parallel; at the most there is a divergence only at the ends of the chromosomes. On deep staining the split may be easily overlooked. The two chromatin nucleoli do not become loose in texture, retain their characteristic red stain with saffranine, and join together in the early synapsis to form one dumbbell-shaped (bivalent) one (*N. 2*, Figs. 4, 5, 8, 10); they do not become longitudinally split like the chromosomes proper. In the early synapsis they are frequently very irregular in form, as I showed in my previous paper, but the apparent fragmentation of them which I then described—a fragmentation of a single long one into two—is not a fragmentation at all, but a stage before the two have joined to form one bivalent one.

Reduction in number of the chromosomes.—In my earlier paper I showed that the number of chromosomes is reduced one-half during the synapsis period—*i. e.*, long before the maturation divisions. I then considered it probable that the reduction in number was effected by a union of chromosomes end to end, but was unable to prove this point. Since then I have been able to demonstrate that this numerical reduction is effected in

the synapsis by the union into seven pairs of the fourteen chromosomes, each of the seven bivalent chromosomes (pairs) being composed of two univalent chromosomes joined end to end (Figs. 5–11). Where the ends of two univalent chromosomes come together is seen a connecting band of linin; each bivalent chromosome during the synapsis and postsynapsis is U- or V-shaped, and the bend or angle of the U or V marks the point of union of two univalent chromosomes; the arms of the U or V are longitudinally split. In each bivalent chromosome only one end of each univalent chromosome is thus closely connected with one end of the other, the opposite ends of the univalent chromosomes having no such linin connections. It has been already mentioned that the two chromatin nucleoli come together likewise to form one bivalent one, and it can be seen that they are connected by a band of linin.

In a paper on the spermatogenesis of *Peripatus* (1901) I showed that it is a particular end of one univalent chromosome which unites with a particular end of another; these ends are the ones which point nearest to the pole of the spindle in the anaphase of the last spermatogonic mitosis, the "central ends," as I have called them, in distinction to the opposite or "distal ends." In *Euchistus*, on the contrary, I am unable to determine positively whether it is similar ends of chromosomes which unite, because in this form the chromosomes have a much more irregular position within the nucleus; the polarity of the nucleus is not so well marked as in *Peripatus*. In the cell body the polarity is as in *Peripatus:* that pole with the greatest amount of cytoplasm and containing the idiozome mass is the distal pole (the one which in the dyaster stage of the last spermatogonic division was in the equator of the cell). This polarity of the cell body is shown in Figs. 4, 5 and 8; I figured it also in a number of cases in my previous paper, but then erroneously supposed the idiozome mass to occupy that point where the spindle pole had previously been, whereas I am now able to determine positively that this pole is situated directly opposite, namely, where the least amount of cytoplasm is situated. Now it would appear in *Euchistus*, though not nearly so regularly as in *Peripatus*, that it is the openings of the U- or V-shaped bivalent chromosomes that are directed toward the distal pole of the cell body (toward the pole where the idiozome mass is placed). Figs. 4, 5 and 8 show this for certain of the chromosomes, while other ones (as two in Fig. 5) may have their openings in opposite directions. Thus in *Euchistus* there is more irregularity in the positions of the axes of the chromosomes, so that I have been unable to determine whether it is, as in *Peripatus*, only particular ends of the univalent chromosomes which unite with particular ends of others.

Throughout the growth period can be seen two kinds of linin threads: (1) thicker threads which connect the ends of the chromosomes, and (2) more delicate ones which join chromatin granules with the nuclear membrane. Apparently, as I have shown for

Peripatus, the former, together with the linin contained in the chromosomes (axial threads), together constitute a single continuous linin spirem in the nucleus.

Rest stage of the spermatocytes.—A rest stage in the growth period preceding the prophases of the maturation mitoses is well marked in *Euchistus* (Fig. 12 and Figs. 95–100 of my preceding paper); though I can confirm Paulmier's observation that such a stage does not occur in *Anasa*. It is characterized by a huge true nucleolus, by a rather diffuse and scattered distribution of the chromatin so that chromosomal boundaries are practically indistinguishable, and by the diffuse arrangement of a great amount of idiozome substance all around the nucleus, so that an idiozome mass with sharp outlines is not present; the idiozome mass in the synapsis stage (Figs. 4, 5, 8), on the contrary, had a sharp and distinct outline. The bivalent chromatin nucleolus has now become nearly rounded in form, rarely showed a dumbbell shape, so that its component parts are very closely apposed. It lies peripheral, in contact with the nuclear membrane, while the true nucleolus lies nearer the centre of the nucleus. Sometimes a much smaller rounded body, staining like the chromatin nucleolus, is also found in the nucleus, but what its origin is I have not been able to determine, for I have not found it in the spermatogonia, though it might well escape detection there on account of its small size.

As to the terminology adopted by me in my former paper (1898) for the series of stages of the growth period, which has been criticised by McClung (1900), the term "metaphase" was, I grant, used by me incorrectly, for I used it for the commencement of the anaphase, whereas it is really Strasburger's stage comparable to Flemming's "metakinesis." However, the exact use of these terms was explained by me (1898, p. 20). In the stages leading up to the resting spermatocyte I distinguished "early anaphase," "synapsis," "postsynapsis," and "telophase" as easily recognizable stages in the growth period of *Euchistus* which need to be characterized by terms for purposes of description. McClung (*l. c.*) considers the appearances of the synapsis stage as artefacts; it is hardly necessary to reply to this criticism, since in all *Metozoa* where the spermatogenesis has been carefully examined, with the exception of certain *Amphibia*, the dense massing of the chromosomes in the synapsis stage has been shown to be a perfectly normal phenomenon. As to my use of "telaphase," Heidenhain's (1894, p. 524) definition is: "Unter dem Namen Telokinesis beschreibe ich gewisse Bewegungen des Kerns und des Mikrocentrums, welche gegen das Ende der Mitose hin stattfinden. . . . Die zugehörigen Stadien der Mitose bezeichne ich als Telophasen." Since Heidenhain employed it for the stage just preceding rest in leucocytes, I was warranted in using it for the stage just before the rest stage in the spermatocytes of *Euchistus*. It must be borne in mind, in the description of the changes of the growth period of the germ cells, that a peculiar stage, the synapsis, occurs, not found elsewhere in mitosis, and that this stage modifies to greater or

less extent, according to the object, the stages which precede and those which succeed. It results from this that the stages of mitosis of the growth period cannot be exactly compared with those of other cells, and hence the terms "anaphase" and "telophase" can here have a significance only approximately similar to that of other mitoses.

The maturation divisions.—In the early prophases the longitudinal splitting of the chromosomes is well marked, clearer than in preceding stages (Figs. 13, 14). Each chromosome is, as before the rest stage, clearly bivalent, formed of two longitudinally split univalent chromosomes joined so as to make an angle together (Figs. 13–15), and at the bend of the angle is a connecting linin thread. These forms of the bivalent chromosomes were clearly figured in my earlier paper, except that then I had overlooked the longitudinal split. The chromosomes gradually become closer, shorter, with smoother outlines, the longitudinal split gradually becomes hidden, and the definitive chromosome with the form of a dumbbell results (Figs. 16–18). In the definitive chromosome there is usually no trace of the longitudinal split, except occasionally as a slight indentation at the free end of a univalent component. The constriction of the dumbbell marks the point of union of two univalent chromosomes, which is effected by a linin band which generally never becomes quite hidden.

In the late prophases, just before the disappearance of the nuclear membrane, and when the centrosome pairs have reached opposite poles of the nuclear surface, a remarkable condition of the linin threads is found (Fig. 17); it was also shown in Figs. 152 and 153 of my earlier paper. The linin, previously in the form of fibres or strands, now takes the form of chains of small globules—quite as Van Beneden (1883) had figured for *Ascaris*. I cannot explain this condition, but I have found it always at this stage, and at this stage only.

In the first maturation division there are seven bivalent chromosomes and one bivalent chromatin nucleolus, and all these elements are divided transversely in metakinesis, being placed in the monaster stage so that their constrictions lie in the plane of the equator. Fig. 18 shows a monaster stage with all these elements on lateral view, Fig. 19 on pole view; this stage was accurately described by me in my former paper, so that I have no additions to make to that description. Whole univalent chromosomes are separated in the ensuing metakinesis, and the univalent components of the chromatin nucleolus are also separated.

When the daughter chromosomes separate in the anaphase a constriction or indentation appears on them (Figs. 193–201 of my preceding paper). This I am now able to prove, in agreement with Paulmier's observations on *Anasa*, is the reappearance of the longitudinal split; this indentation or constriction becomes placed in the equatorial plane of the monaster stage of the second maturation division, so that the latter division divides

the chromosomes equatorially. In the anaphase of the first maturation division the constriction of the chromosomes generally has the appearance shown in Fig. 195 of my former paper; while Fig. 196, which I then considered to represent the typical condition, I now, from the study of more abundant material, find to be an unusual condition. That is to say, the appearance of the chromosomes shown in Fig. 196 of my preceding paper is really atypical, since in this case their constrictions appear at right angles to the long axis of the spindle, whereas in most other cases the planes of these constrictions coincide with planes passing through the long axis of the spindle. In this second maturation division the chromatin nucleolus is not always divided.

2. *Euchistus tristigmus* Say

Four testes of this species were studied.

In the rest stage of the spermatogonium there are two small chromatin nucleoli, generally attached to the surface of the true nucleolus.

In the spermatogonic mitosis there are fourteen chromatin segments in the equatorial plate (Pl. I, Fig. 20); the twelve larger, usually somewhat elongate ones are chromosomes, and the two smallest, rounded ones are chromatin nucleoli. All these elements are halved in metakinesis.

In the synapsis stage the twelve chromosomes unite to form six bivalent chromosomes. The two chromatin nucleoli sometimes unite to form a bivalent one, which is clearly dumbbell-shaped in earlier stages, but in the resting spermatocyte becomes rounded and has a peripheral position (Fig. 22); or quite as frequently they remain separate from one another during the growth period, and are seen to be of unequal volumes (Fig. 21). The chromatin nucleoli in the growth period are rarely attached to the true nucleolus.

In the first maturation division there are always six clearly bivalent, dumbbell-shaped chromosomes and either one dumbbell-shaped bivalent chromatin nucleolus or, apparently more frequently, two univalent chromatin nucleoli of more or less rounded form and different volume (lateral view shown in Fig. 25, N. 2). Accordingly, on pole views of the monaster stage there are seen either seven chromatin elements (Fig. 24), which are six bivalent chromosomes and one bivalent chromatin nucleolus, or there are eight, namely, six bivalent chromosomes and two univalent chromatin nucleoli (Fig. 23: in this figure one of the chromatin nucleoli can be distinguished by its smaller size, but which of the remaining seven elements is the other chromatin nucleolus is not easily discernible on pole views, since the larger of the two chromatin nucleoli has a diameter equal to that of one of the smaller chromosomes).

All the six chromosomes are halved (by a reduction division) in metakinesis, so that

in the monaster stage of the second division there are six univalent chromosomes, the constrictions of which represent the reappearance of the longitudinal split (Fig. 26); there are also in the same equatorial plate two non-constricted bodies of different volumes which are not joined together. These are the chromatin nucleoli, which are regularly halved in the first maturation metakinesis—that is, they are the halves of univalent ones. Thus the bodies marked *N. 2* in Fig. 26 are the halves of those similarly marked in Fig. 25.

The second maturation division is equatorial, and the spermatid receives six chromosomes, arranged in an outer circle around a single central chromatin nucleolus. Accordingly in this second division one chromatin nucleolus passes undivided into one daughter cell (spermatid), the other undivided into the other daughter cell.

As in *Euchistus variolarius*, two follicles of the testis contain spermatocytes of a much larger size than those in the four other follicles.

3. *Podisus spinosus* Dall.

Five testes of this species were studied.

In the spermatogonic rest stage there are two small chromatin nucleoli, of more or less rounded form, attached to the surface of a true nucleolus.

In the spermatogonic monaster there are sixteen chromatin segments (Pl. I, Fig. 27), two of which probably correspond to the chromatin nucleoli of the previous rest stage.

In the synapsis the fourteen chromosomes unite to form seven bivalent chromosomes. The two chromatin nucleoli also come together to make one bivalent one; in the growth period of the spermatocytes (Fig. 28) the chromatin nucleolus lies close to the nuclear membrane, and to its inner surface the true nucleolus is regularly attached.

In the first maturation monaster there are eight chromatin elements, namely, seven chromosomes and one chromatin nucleolus, all bivalent and dumbbell-shaped on lateral view; the chromatin nucleolus has about the same volume as the smaller ones of the chromosomes, and so cannot be distinguished from them with certainty.

4. *Mormidea lugens* Fabr.

Five testes were studied.

In the rest stage of the spermatogonia there are two chromatin nucleoli (Pl. I, Fig. 30, *N. 2*), which may be equal or unequal in size; they may be attached together, which is apparently the general rule, or may be separated, and one or both of them may be apposed to the true nucleolus.

In the spermatogonic monaster there are sixteen chromatin segments; two of these

which are smaller than the others and more rounded are the chromatin nucleoli (Fig. 31, *N. 2*).

In the synapsis the fourteen chromosomes unite to form seven bivalent ones, and the two chromatin nucleoli to form one bivalent chromatin nucleolus. The latter is peripherally placed in the nucleus, and not attached to the true nucleolus (Fig. 32).

In a pole view of the monaster stage of the first maturation division are found eight chromatin elements (Fig. 33); lateral view shows all are bivalent and dumbbell-shaped, seven are chromosomes, and one easily recognizable by its much smaller size in the chromatin nucleolus (Fig. 33, *N. 2*).

5. *Peribalus limbolaris* Stal.

Two testes of this species were studied.

In the rest stage (Pl. I, Fig. 34) and prophases of the spermatogonia are found two chromatin nucleoli of unequal size (*N. 2*), and sometimes apparently three; they are generally not in mutual contact, though they are often apposed to the true nucleoli, of which there are frequently two or three.

In the spermatogonic monaster (Fig. 35) are sixteen chromatin segments, of which the two smallest, rounded ones are the chromatin nucleoli; the fourteen chromosomes are notably elongated.

In the synapsis the fourteen chromosomes unite to form seven bivalent ones, and the two chromatin nucleoli to form one bivalent chromatin nucleolus; in the rest stage of the spermatocytes (Fig. 36) the chromatin nucleolus (*N. 2*) is usually rounded and peripherally placed, and generally unattached to the relatively very large true nucleolus (sometimes there are two true nucleoli, as in Fig. 36, rarely three). In the rest stage of the spermatocytes there is a smaller chromatin nucleolus in addition to the larger one already described, and this smaller may correspond to the third of the chromatin nucleoli found sometimes in the rest stage of the spermatogonia.

In the monaster stage of the first maturation division there are eight chromatin segments (Fig. 37), namely, seven bivalent, dumbbell-shaped chromosomes and one much smaller bivalent, dumbbell-shaped chromatin nucleolus.

6. *Cosmopepla carnifex* Fabr.

Five testes of this species were studied.

In the monaster stage of the spermatogonia (Pl. I, Fig. 38) are found eighteen chromatin segments; two of these are smaller than the others, and so by analogy with other species of this family probably represent chromatin nucleoli (*N. 2*, Fig. 38); the sixteen other segments are then true chromosomes.

In the synapsis stage of the growth period the sixteen chromosomes unite to form eight bivalent chromosomes, and the two chromatin nucleoli to form one bivalent chromatin nucleolus. The latter is, in the rest stage of the spermatocytes, rounded and peripheral in position, and is not attached to the larger true nucleolus (Fig. 39); both the nucleolus and the chromatin nucleolus may contain a large, clear vacuole, which in the former is excentric.

Pole views of the monaster stage of the first maturation division show nine chromatin elements (Fig. 41), and lateral views (Fig. 40) of the same stage show that all are bivalent and dumbbell-shaped. The smallest of these elements is the chromatin nucleolus (*N. 2*).

7. *Nezara hilaris* Say

Five testes of this form were studied.

There are in the rest stage and early prophases of the spermatogonia two chromatin nucleoli, which are comparatively large and usually more or less unequal in size (Pl. I, Figs. 42, 43, *N. 2*). They are generally peripheral in position and in mutual contact, but usually are not apposed to the true nucleolus (*N*).

In the spermatogonic monaster there are sixteen chromatin segments (Fig. 44), of which two can be always recognized by their small size and rounded form as the chromatin nucleoli; the fourteen chromosomes are generally elongated.

In the synapsis the two chromatin nucleoli unite to form one bivalent one, and apparently also the fourteen chromosomes join to make seven bivalent chromosomes, but I cannot state this with certainty. In the telophase of the spermatocytes (Fig. 45), the chromatin nucleolus (*N. 2*) is peripherally placed and clearly bivalent, and usually not in connection with the very large true nucleolus (*N*), which is also peripheral.

In the testes examined (all from individuals secured in the month of September) were stages only from the resting spermatogonia to the telophase of the spermatocytes; all later stages in the spermatogenesis were absent, so that the number of the chromosomes in the maturation divisions could not be determined.

The longitudinal split in the chromosomes during the growth period is unusually distinct in this species.

8. *Brochymena* sp.

Three testes of this species were studied.

In the rest stage of the spermatogonia (Pl. I, Fig. 46) are two small chromatin nucleoli (*N. 2*), which are peripheral in position, of nearly equal size, generally mutually apposed, and seldom attached to the true nucleolus.

In the spermatogonic monaster stage (Pl. I, Fig. 47) are sixteen chromatin segments, of which two are smaller and rounded and are the chromatin nucleoli.

In the synapsis stage the fourteen chromosomes unite to form seven bivalent ones, and the two chromatin nucleoli to form one bivalent chromatin nucleolus. The latter in the stages following the synapsis is rounded and peripheral in position (*N. 2*, Fig. 48, Pl. II), and only occasionally attached to the true nucleolus (*N*).

Pole views of the first maturation monaster (Pl. II, Fig. 49) show eight chromatin segments, of which one easily distinguishable from the others by its smaller size is the chromatin nucleolus (*N. 2*). Lateral views of this stage show that all eight of these elements are bivalent and dumbbell-shaped.

9. *Perillus confluens* H.-S.

Two testes of this species were examined.

The rest stage of the spermatogonia (Pl. II, Fig. 50) shows two small, rounded chromatin nucleoli of unequal size, which are always attached together, and may be either close to the nuclear membrane or apposed to the surface of a true nucleolus (*N*).

In the monaster stage of the spermatogonic divisions are sixteen chromatin segments (Fig. 51). The fourteen largest are chromosomes, the two smallest are chromatin nucleoli (*N. 2*); the latter are more minute than in the corresponding stage of any other Pentatomid examined by me, and on account of their small size cannot always be seen (*i. e.*, in cases where they are closely apposed to the chromosomes).

In the synapsis stage the fourteen chromosomes unite to form seven bivalent ones and the two chromatin nucleoli to make one bivalent chromatin nucleolus. The latter is dumbbell-shaped in the earlier stages of the growth period, but in the rest stage (*N. 2*, Fig. 52) becomes oval in outline, and it is then attached to the surface of the larger true nucleolus (*N. 2*), the two occupying a more or less central position within the nucleus.

Pole views of the monaster stage of the first maturation division show eight chromatin segments of varying diameter (Fig. 53); one of these, probably the smallest, is the chromatin nucleolus; lateral views show that all these elements are bivalent and dumbbell-shaped.

10. *Cœnus delius* Say

Three testes of this species were studied.

In the rest stage of the spermatogonia there are two chromatin nucleoli with irregular outlines (Pl. II, Fig. 54, *N. 2*), and they are situated usually close together.

In the spermatogonic monaster stage (Fig. 55) there are fourteen chromatin segments, the two smallest of which are probably the chromatin nucleoli (*N. 2*), leaving twelve chromosomes.

In the synapsis stage the twelve chromosomes unite to form six bivalent ones. The

two chromatin nucleoli found in the spermatogonia also unite to form one bivalent chromatin nucleolus; this is clearly bilobed in the earlier stages, but more rounded in the later stages of the growth period of the spermatocytes (the larger of the bodies designated *N. 2* in Figs. 57, 58, 63); there is attached to it usually a small true nucleolus (Fig. 58). Besides this large bivalent chromatin nucleolus there is also found in the spermatocytes, most clearly seen in the rest stage, another much smaller one, of rounded form (the smaller of the bodies marked *N. 2* in Figs. 57, 58, 63); this is almost always apposed to one of the true nucleoli (*N*), of which there are generally two large ones besides the small one attached to the large chromatin nucleolus; quite frequently the small chromatin nucleolus lies between the large one and a large true nucleolus (Fig. 63). This small chromatin nucleolus is difficult to see in the synapsis stage, when the chromosomes stain deeply, and since I was also unable to find it in the monaster stage of the spermatogonia, I could not determine whether it is bivalent or univalent or what its earlier history is. It might well be present, however, in the spermatogonia, but be there so minute as to escape detection.

Pole views of the monaster stage of the first maturation division show sometimes only seven chromatin segments (Fig. 62), and then these are six bivalent chromosomes and the large bivalent chromatin nucleolus; or they show eight segments (Figs. 59, 60), of which the smallest is the small chromatin nucleolus of the growth period. That is to say, in the equatorial plate there are always six bivalent chromosomes and the large bivalent chromatin nucleolus, while the small chromatin nucleolus may be present or may be absent. The lateral view of this stage given here (Fig. 61) shows seven large dumbbell-shaped elements, ot which six are chromosomes and one the bivalent chromatin nucleolus—though which one it would be hard to say, for all of these elements are of approximately equal size and similar form; while the smallest, eighth, element marked *N. 2* in this figure is the small chromatin nucleolus. When the latter persists into this stage it appears to be halved in the following metakinesis.

II. *Trichopepla semivittata* Say

Four testes of this species were studied.

In the nucleus of the resting spermatogonium are seen clearly two rounded chromatin nucleoli (*N. 2*, Fig. 64, Pl. II), of different volumes, one or both frequently apposed to a larger true nucleolus (*N*).

In the monaster stage of the spermatogonia are found sixteen chromatin segments, of which fourteen are elongate chromosomes, and two which are smaller and rounded are the chromatin nucleoli (*N. 2*, Fig. 65), which here, as in the preceding rest stage, are unequal in size.

In the following synapsis stage the fourteen chromosomes join to form seven bivalent ones. The two chromatin nucleoli likewise unite to form one bivalent one, of which the two components are unequal in size (Fig. 66, *N. 2*). In the telophase and rest stage of the spermatocytes the chromatin nucleolus loses its earlier bipartite form and becomes rounded (the larger of the bodies marked *N. 2* in Fig. 67), and only occasionally is it apposed to the larger true nucleolus (*N*). Sometimes the two chromatin nucleoli derived from the spermatogonia do not unite together, but remain separated. During the growth period, its later stages at least, can be seen in each nucleus three or four much smaller, rounded bodies, which stain like the chromatin nucleoli; some of them are often attached to the surface of the true nucleolus (Fig. 67, the three smaller bodies designated *N. 2*). There are certainly three of them and in some nuclei apparently four. I was unable to determine with certainty these small chromatin nucleoli in the rest and division stages of the spermatogonia, though they might well be present there, but escape observation on account of their minuteness.

The monaster stage of the first maturation division (Figs. 68, 69) shows eight larger, bivalent, dumbbell-shaped chromatin segments, of which seven are chromosomes and one the large chromatin nucleolus (*N. 2* of the figures). Of the seven chromosomes one is always longer and more voluminous than the others (Figs. 68, 69), and is probably the derivative of the two largest chromosomes found in the spermatogonic divisions (Fig. 65). Besides these eight large elements of the monaster stage of the reduction division there may be seen on pole view usually one (Fig. 68), sometimes two much smaller granules, which evidently represent the small chromatin nucleoli found in the growth period.

SCUTELLARIIDÆ.

12. *Eurygaster alternatus* Say

Three testes of this form were studied from individuals taken in July and August. Each testis was filled with spermatocytes and spermatids, but contained no spermatogonia.

In the spermatocyte in the rest stage one bilobed and hence probably bivalent chromatin nucleolus (*N. 2*, Fig. 70, Pl. II), which is peripheral in position and separated from the usually smaller true nucleolus (*N*). Sometimes the two components of this chromatin nucleolus do not join together in the synapsis but remain separated through the growth period.

In the monaster stage of the first maturation division (Fig. 71, pole view) are found seven dumbbell-shaped (and hence probably bivalent) chromatin segments, of which the smallest is undoubtedly the chromatin nucleolus (*N. 2*), so that here there would be six bivalent chromosomes.

COREIDÆ.

13. *Anasa tristis* De G.

Twenty-one testes of this species were studied.

In regard to the chromosomal numbers my observations confirm those of Paulmier.

In the rest stage of the spermatogonium (Pl. II, Figs. 72, 73) there are two chromatin nucleoli (*N. 2*), which are much smaller than the true nucleoli (*N*) to which they are generally apposed. They have definite irregularly rounded or oval outlines as examined with Hermann's saffranine-gentian violet stain, and are not "hazy" or "indefinite" as Paulmier (1899) described. Both may be attached to the same nucleolus, or they may be joined to separate nucleoli. Sometimes each one may separate into two pieces (as is the case with one in Fig. 72). They are best seen on iron hæmatoxylin preparations so strongly destained that the chromatin reticulum does not appear.

In the monaster stage of the spermatogonia (Fig. 74) are twenty-two chromatin segments, namely, twenty larger chromosomes and two much smaller chromatin nucleoli (*N. 2*).

In the synapsis stage the twenty chromosomes unite to form ten bivalent ones, and the two chromatin nucleoli to form one bivalent one. The latter is clearly bipartite in the synapsis, but later shows an oval outline (Fig. 75); it is peripheral in position, often contains a central clearer vacuole as in the *Pentatomidæ*, and is as a rule separated from the true nucleolus (*N*).

In the monaster stage of the first maturation division (pole view, Fig. 76) are found eleven bivalent, dumbbell-shaped chromatin segments, of which the central, smallest one is the chromatin nucleolus (*N. 2*). I am able to confirm Paulmier's (1899) account of the two maturation divisions.

14. *Anasa armigera* Say

One testis of this species was studied.

The spermatogenesis seems to be very similar to that of the preceding species, but as I had no preparation stained with saffranine-gentian violet I was unable to determine the relations of the chromatin nucleoli in the rest stage of the spermatogonia.

Fig. 77, Pl. II, shows a pole view of a monaster stage of the spermatogonia, with twenty chromosomes and two chromatin nucleoli (*N. 2*); it is very similar to the corresponding stage in *Anasa tristis* (Fig. 74).

In the synapsis are formed ten bivalent chromosomes and one bivalent chromatin nucleolus.

In the monaster of the first maturation division (Fig. 78) are ten bivalent chromo-

somes and one bivalent chromatin nucleolus; Fig. 78 is a pole view, but in it those elements which appear dumbbell-shaped are seen from the side.

15. *Anasa* sp.

Of this undetermined species, which was collected for me at Berryessa in California I examined nine testes.

The resting spermatogonium shows two chromatin nucleoli (Pl. II, Fig. 79, *N. 2*) which are comparatively large and rather loose in texture, generally irregular in outline, occasionally attached to the true nucleolus (*N*), and more or less central in position.

In the monaster stage of the spermatogonia (Fig. 80) are twenty-two chromatin segments, namely, twenty larger chromosomes and two smaller chromatin nucleoli (*N. 2*).

In the synapsis the chromosomes unite to form ten bivalent ones, and the chromatin nucleoli to form one bivalent one. In the rest stage of the spermatocytes (Fig. 81) the chromatin nucleolus (*N. 2*) is seen to be somewhat elongate in form, is peripheral in position, and not attached to the true nucleolus (*N*).

In the monaster stage of the first maturation division are eleven bivalent elements, of which the smallest is the chromatin nucleolus (*N. 2*, Fig. 82); in this figure we do not have strictly pole views of all the chromosomes.

Fig. 83 shows four of the bivalent chromosomes on lateral view, in a paratongential section of a cell in the stage of the first maturation monaster. It is given here because it is the clearest case I have noticed in any Hemipteron of the quadripartite nature of these chromosomes, for while the transverse split may generally be seen at this stage, the longitudinal split is generally hidden. The poles of the spindle (not in the plane of this section, but seen in the next one to it) are situated at the upper and lower portions of the figure respectively; and it is hardly necessary to add that the first maturation division coincides with the plane of the transverse split, the second with the plane of the longitudinal split.

16. *Metapodius terminalis* Dall.

Eleven testes were studied of this species, which is very favorable on account of the large size of the cells; one should examine testes from individuals taken in June or early July, before the time of copulation.

In the rest stage of the spermatogonia (Pl. II, Fig. 84) are two chromatin nucleoli (*N. 2*) of very small size and smooth outlines, generally close together on the surface of a true nucleolus (*N*).

In the spermatogonic monaster (Fig. 85) are twenty-two chromatin segments, of which the two smallest are chromatin nucleoli (*N. 2*) and easily recognizable.

In the synapsis the twenty chromosomes unite to form ten bivalent ones, and the two chromatin nucleoli to form one bivalent one. The latter is in later stages of the growth period bilobed (*N. 2*, Fig. 86), is peripheral in position and not opposed to the larger true nucleolus (*N*).

In the monaster stage of the first maturation division are eleven chromatin segments (Fig. 87), of which the smallest, centrally placed one is the chromatin nucleolus (*N. 2*); all these elements are bivalent and on lateral view they all show the dumbbell-shape.

17. *Chariesterus antennator* Fabr.

Two testes of this form were examined.

In the rest stage of the spermatogonia I could not be certain of the presence of chromatin nucleoli, for my preparations were not very well stained to demonstrate them. There were also no spermatogonic monasters favorable enough for determining the number of chromosomes.

In the synapsis stage there is a bivalent chromatin nucleolus, but sometimes its component parts are widely separated.

In the telophase of the spermatocytes (Pl. II, Fig. 88) the chromatin nucleolus (*N. 2*) is peripheral in position, sometimes its two univalent components still separated (but that is not the case in Fig. 88). The true nucleolus (*N*) is sometimes central, sometimes peripheral in position, and occasionally it is apposed to the chromatin nucleolus.

In the monaster of the first maturation division (Fig. 89, lateral view; Fig. 90, pole view) are found thirteen chromatin segments, of which the smallest, centrally placed one is the bivalent chromatin nucleolus (*N. 2*). Of the twelve chromosomes at least eleven would seem to be bivalent (having the characteristic dumbbell-shape); but in the lateral view here given (Fig. 89), it will be noted that the chromosome nearest the left-hand side does not appear dumbbell-shaped. This may be a bivalent one seen obliquely, or it may be a univalent one; which is the case I cannot determine, since there were few satisfactory lateral views on the preparations and since the number of chromosomes in the spermatogonia could not be determined.

18. *Alydus pilosulus* H. S.

Four testes of this species were studied.

The chromatin nucleoli in the rest stage of the spermatogonia (*N. 2*, Fig. 91, Pl. II) are two in number and rounded; they are very small, usually close together, and may be or not be attached to the true nucleolus (*N*).

In the spermatogonic monaster stage (Fig. 92) are fourteen chromatin segments, two

of which, easily distinguishable from the others by their small size, are chromatin nucleoli (*N. 2*).

In the synapsis stage the twelve chromosomes unite to form six bivalent ones, and the two chromatin nucleoli to form one bivalent one. In the late stages of the growth period (Figs. 93, 94) the chromatin nucleolus (*N. 2*) is rounded and peripheral in position, and usually apposed to the true nucleolus; even when they are separated the latter is usually peripheral (*N*, Fig. 94)—an unusual position for it in spermatocytes of *Hemiptera.*

In the monaster stage of the first maturation division (Fig. 95) are seven elements, namely, six bivalent chromosomes and one bivalent chromatin nucleolus (the smallest of the seven elements, *N. 2*); all these are dumbbell-shaped on lateral view, and though Fig. 95 is a pole view of the spindle two of its chromosomes are seen from the side.

19. *Alydus eurinus* Say

One testis of this species was studied.

In the rest stage of the spermatogonia I could not determine chromatin nucleoli, probably on account of their small size.

Numerous monaster stages of spermatogonia were examined, and all showed thirteen chromatin elements (Pl. III, Fig. 96); two of these which are readily recognizable from the others by their minute size are chromatin nucleoli (the two small granules shown in Fig. 96); the eleven large elements are chromosomes, and have mostly an elongated form.

In the synapsis the two chromatin nucleoli unite to form one bivalent one, which in the telophase of the spermatocytes (*N. 2*, Fig. 97) is relatively small, peripheral in position, and quite frequently apposed to the larger true nucleolus (*N*). Of the eleven univalent chromosomes derived from the spermatogonium, ten unite to form five bivalent pairs, while one (the eleventh) does not unite with any of the others but remains univalent.

In the first maturation division are found seven chromatin elements (Fig. 98, pole view); the smallest of these is bivalent, dumbbell-shaped, and is the chromatin nucleolus (*N. 2*); the six larger elements are chromosomes. Now a careful study of numerous monaster stages seen on lateral view shows that only five of these chromosomes are dumbbell-shaped, and so bivalent on analogy with what is known for the other *Hemiptera;* while one of them is never dumbbell-shaped, approximately half the volume of the others, and is univalent. Fig. 99 is a lateral view of the spindle of the first maturation division, showing three of the five dumbbell-shaped chromosomes and (most to the right) the univalent chromosome. In all cases where the chromosome plates of this stage can

be seen on lateral view, one chromosome is always found to be of about half the size of the others and not dumbbell-shaped; on pole views this chromosome can be distinguished by its lesser depth.

So in *Alydus eurinus* there is an uneven number of chromosomes in the spermatogonia, namely, eleven; the reduction in number is effected then in the synapsis by ten combining to form five bivalent ones, while one remains univalent and uncombined, because there is no mate with which it can unite.

In the monaster of the second maturation division there are either six or seven chromatin elements. In Fig. 100 of this stage are shown seven, of which the smallest is probably the chromatin nucleolus, five are halves of the originally bivalent chromosomes and one probably the half of the originally univalent chromosome.

In the spermatid we find either six (Fig. 102) or five (Fig. 101) chromatin elements of approximately equal volume. Now these elements are too large to be derivatives of the chromatin nucleolus of the spermatocyte of the first order (*N. 2*, Fig. 98), so that the five or six elements of the spermatids would not seem to represent portions of this chromatin nucleolus; very probably the latter is so small in the spermatids or generally so closely applied to the surface of one of the chromosomes that it escapes observation. If we then eliminate the possibility of any of the elements shown in the spermatids (Figs. 101, 102) representing chromatin nucleoli or their derivatives, then we must conclude that the five or six elements here are chromosomes. But why is their number sometimes five, in other cases six? Now we know that in all other *Hemiptera* in which attention has been given to this point that each spermatid receives one-quarter of each of the bivalent chromosomes present in the spermatocyte of the first order. Accordingly it would be probable by analogy that in *Alydus eurinus* the spermatid receives one-quarter of each of the original five bivalent chromosomes. Then in the case of Fig. 101 all five elements would be such derivatives; in Fig. 102, five of the six elements. The sixth element of Fig. 102 is then probably the original univalent chromosomes of the first maturation division, which in either the first or the second maturation division could not have been divided, but must have passed undivided into one of the daughter cells; this would explain why sometimes there are only five, sometimes six chromosomes in the spermatid, for, as I have explained, none of the elements of Figs. 101 and 102 can be regarded as chromatin nucleoli.

Of course the preceding is only an attempt at a right interpretation; I have not been able to follow the univalent chromosome with precision in regard to its behavior in the maturation divisions.

20. *Corizus lateralis* Say

Four testes of this species were studied.

I could not determine whether there are chromatin nucleoli in the rest stage of the

spermatogonia, and none of the cases of spermatogonic monasters in my preparations were sufficiently favorable to allow accurate counting of the chromatin elements.

In the growth period of the spermatocytes a comparatively small, bivalent chromatin nucleolus, which in the rest stage (*N. 2*, Fig. 103, Pl. III) has a peripheral position and rounded form and is not apposed to the true nucleolus (*N*). Besides this chromatin nucleolus one or two much smaller, rounded ones can sometimes be seen in the nuclei of the resting spermatocytes, and these stain like the large one with the double stain of Hermann.

In the monaster stage of the first maturation division are always found at least seven chromatin elements; when there are eight the eighth is a small granule (Fig. 105, the smaller of the bodies marked *N. 2*), and this small element, which frequently cannot be seen at this stage, probably represents one of the minute chromatin nucleoli of the growth period. Of the seven larger elements the smallest, centrally placed one is the bivalent chromatin nucleolus (*N. 2*, Fig. 104, and the larger of the elements marked *N. 2* in Fig. 105); this chromatin nucleolus often has its component halves separated (except for a joining linin band) before the period of metakinesis (Fig. 106). The remaining six elements are chromosomes, and of them four are of approximately equal volume, while one is always much larger and one always much smaller than these four (pole views Figs. 104, 105, lateral view Fig. 106). The five largest chromosomes are clearly dumbbell-shaped on lateral view, and accordingly by analogy with the corresponding elements of other *Hemiptera* may be considered bivalent, even though the number in the spermatogonia was not determined. But the smallest chromosome puzzled me at first with regard to its valence, for it is not more than half the volume of the other five, and sometimes it does not appear dumbbell-shaped, so that I considered the possibility of its being univalent; but a careful study of it in numerous cells of the first maturation division resulted in showing in a number of clear cases that it is transversely constricted even before it becomes arranged in the plane of the equator, so that there can hardly be a doubt as to its being bivalent. Fig. 106 shows such a case in an oblique lateral view of the spindle before the chromosomes have become arranged all in one plane, with a well-marked constriction of the smallest chromosome.

The first maturation division is a reduction division and always halves the six chromosomes and the bivalent chromatin nucleolus.

21. *Harmostes reflexulus* Stal.

Thirteen testes of this species were studied.

In the rest stage of the spermatogonia there are two rounded chromatin nucleoli (*N. 2*, Fig. 107, Pl. III), of which one or both may be apposed to the true nucleolus (*N*).

In the monaster stage of the spermatogonic divisions can always be counted thirteen chromatin segments on favorable pole views—*i. e.*, in such cases where these elements can be seen all in one plane, and where they are not too closely apposed to one another (Figs. 108–110). Two of these elements are always distinguishable from the others by their smaller size and rounded shape, and these are the chromatin nucleoli (*N. 2*); they may lie close together (Fig. 110), but more usually are more separated in position (Figs. 108, 109). The remaining eleven elements, which are of large size and elongate form, are chromosomes. There can be no doubt that this is the actual number of these chromosomes, for no exceptions to it were found, and in fourteen clear cases from four different testes the number eleven was obtained with great clearness; these chromosomes are larger than the spermatogonic chromosomes of any other Hemipteron examined. Sometimes one or more of the chromosomes may show a slight transverse constriction, but this is not a constant appearance.

In the synapsis the two chromatin nucleoli derived from the spermatogonium unite to form one bivalent one; in the rest stage (which is very complete) of the spermatocyte it is elongate (*N. 2*, Fig. 111), peripheral in position and not attached to the true nucleolus; the latter is larger (*N*, Fig. 111), frequently peripheral in position, and sometimes two true nucleoli are present.

During the synapsis stage ten of the eleven chromosomes join to form five bivalent chromosomes, while the eleventh remains univalent, as will become evident from the following description:

In the first maturation division are found either seven chromatin elements or eight chromatin elements; these two conditions may be described successively.

When there are seven elements (Fig. 112 pole view of the monaster stage, Fig. 113 lateral view) one may always be distinguished by its smaller size and central position, and by its history from the rest stage of the spermatocyte to the stage under discussion this is found to be the chromatin nucleolus (*N. 2*); this we have already learned to be bivalent, and in Fig. 113 its univalent components are seen to be separating. The six larger elements are chromosomes. Five of these, as Fig. 113 shows, are clearly dumbbell-shaped and bivalent. The sixth, however (*x*), never shows a dumbbell shape before the metakinesis, but is always distinguishable from the others by its oval form. From these appearances we must conclude that this sixth chromosome is univalent—represents the odd, eleventh, chromosome of the spermatogonic monaster stage, which had no mate with which to unite during the following synapsis stage. It was in this species that I first found spermatogonia with an uneven number of chromosomes, so that I first concluded they must be abnormal cases, for heretofore in all objects the spermatogonic (normal) number has been described as an even one; I immediately sectioned testes of other

individuals to determine this point, but, as has been already stated, in the four of the thirteen testes which contained spermatogonic divisions exactly eleven chromosomes were found to be always present. Obviously all of the eleven chromosomes cannot unite into pairs during the synapsis, one must remain unmated, and this must be necessarily that one of the first maturation division which does not appear bipartite.

Now in those cases where there are eight chromatin elements present in the spermatocytes the question becomes more complicated (Figs. 114–116). Here, as in the cases where there are seven elements, one is central in position and distinguishable from the others by its smaller volume, namely, the bivalent chromatin nucleolus (*N. 2*, Figs. 114–116, in the last figure its univalent components separated); the remaining seven elements are then chromosomes. As the lateral view, Fig. 116, of the spindle shows, four of these are bivalent (the ones not marked by lettering). One (*x*) is oval in form, showing no constriction or splitting, and so is probably comparable to the univalent chromosome of those cells which contain but seven chromatin elements (*i. e.*, to *x* in Fig. 113.) There then remain the two elements marked *a* in Fig. 116, and each of these I conclude must be a univalent chromosome, which in cases where there are only seven chromatin elements in the spindle would have combined with the other to form one bivalent chromosome; if this be so, then the transverse constriction of the left-hand chromosome marked *a* in Fig. 116 would not be the line of separation between two univalent chromosomes. Another reason for looking upon these two chromosomes as univalent, is because they are of approximately the same volume as the chromosome marked *x*, which we have shown to be univalent by comparison with the chromosome *x* of Fig. 113. But there is a still better reason for considering the elements *a* of Fig. 116 to be univalent chromosomes. A pole view of a corresponding stage with eight chromatin elements shows the seven chromosomes frequently equidistant from one another (as in Fig. 115). But often we find on pole view two of the seven chromosomes close together and connected by a band of linin (*a*, Fig. 114); the two together constitute a virtual bivalent chromosome, which, however, differs from the other bivalent ones in having its long axis parallel to the plane of the equator of the spindle. Let the band of linin which connects these chromosomes *a* become stretched out, and as a result we would have a bivalent chromosome lying parallel to the plane of the equator, and with its univalent halves widely separated—*i. e.*, the condition that maintains for the chromosomes *a* of Fig. 116.

To summarize, we find two conditions in the spermatocytes: (1) there are seven chromatin elements, namely, one bivalent chromatin nucleolus, five bivalent chromosomes, and one univalent chromosome; and (2) eight chromatin elements, namely, one bivalent chromatin nucleolus, four bivalent chromosomes, one univalent chromosome (corresponding to that of condition 1), and two other univalent chromosomes (which

together would correspond to the fifth bivalent chromosome of condition 1). To determine which of these conditions is the more usual, I counted the number of chromatin elements seen on pole views of the monaster stage of the first maturation division. These counts were made on spermatocytes from five different testes, and may be condensed into the following table:

PREPARATION NO.	EIGHT ELEMENTS.	SEVEN ELEMENTS.
86	4	33
238	6	12
408	2	1
410	1	9
356	0	23
Total =	13	78

Thus those spermatocytes with seven chromatin elements would seem to be the more frequent condition. In both cases there is one univalent chromosome, which represents the odd chromosome of the spermatogonia; but why in cells of the second condition two chromosomes should remain separated instead of combining to form a bivalent one, as they do in the first condition, I cannot explain, unless perhaps the presence of the odd univalent chromosome may in some way disturb the union into pairs of the ten other chromosomes during the synapsis.

In those cases where there are seven chromatin elements in the equator of the first maturation spindle, the metakinesis results in the division of all the elements; this is a reduction (transverse) division of the bivalent chromatin nucleolus and of the five bivalent chromosomes, but in what plane the univalent chromosome divides could not be determined. Only one case was seen where the univalent chromosome was left undivided in the equator after the daughter elements of the six other elements had reached opposite poles of the spindle. Thus it would seem that in this division, in the cases where there are seven elements present, all the elements become divided; how it is in the cases where there are eight elements could not be determined. In the spermatid are found either six chromosomes (Fig. 117) and one chromatin nucleolus (*N. 2*), or five chromosomes and one chromatin nucleolus. This would show that the chromatin nucleolus and five chromosomes (the derivatives of the original five bivalent ones) divide in the second maturation division, but that the sixth chromosome, the derivative of the originally univalent one, does not divide but passes undivided into one of the two spermatids. Thus the valence of the seven elements in these generations would be: *first spermatocyte*, one bivalent chromatin nucleolus, five bivalent chromosomes, one univalent chromosome;

second spermatocyte, one univalent chromatin nucleolus, five univalent chromosomes, one semivalent chromosome; *spermatid*, one semivalent chromatin nucleolus and either five or six semivalent chromosomes.

22. *Protenor belfragei* Hagl.

Five testes of this exceedingly interesting species were studied from individuals that had just completed their last ekdysis.

In the rest stage of the spermatogonia (Pl. III, Fig. 118) are two rounded chromatin nucleoli which are usually attached to the surface of a much larger true nucleolus (*N*).

Pole views of the monaster stage of the spermatogonic mitosis show with great distinctness exactly thirteen chromatin elements (Figs. 119–123). This number was found in thirteen cells of one testis, in about sixteen cells of a second, in six cells of a third, and in two cells of a fourth—these being all the favorable cases found, and all these testes had been fixed with Flemming's fluid (the stronger mixture). The fifth testis sectioned had been fixed in piero-acetic acid, and in it the number of chromosomes could not be counted because of the swelling action which this reagent exerts upon the chromatin. These chromosomes are unusually large and on suitable preparations can be counted with exactness. In only two of the cells in which they were counted was there observed a fourteenth element; this was a minute granule (*t*, Fig. 121), which, on account of its being present so rarely in these monaster stages and on account of there being no element to represent it in the later history of the spermatogenesis, need not be taken into account; it seems to be very inconstant, and might possibly represent either a portion of chromatin which had become separated from one of the chromosomes, or a chromatin nucleolus transmitted from some distant parent and now nearly reaching disappearance.

Which two of the elements in the spermatogonic monaster represent the chromatin nucleoli of the previous rest stage I am unable to determine, but that two of them do represent these bodies there can be no doubt from what has been determined for the succeeding stages; judging by analogy with the case in all other *Coreidæ* examined, they would probably be the two smallest elements. Now Figs. 119–123 show what is to be seen very distinctly in all cases, namely, that there are three chromatin elements much larger than the ten remaining. One of these three, that designated *x* in Figs. 119–123, imposes by its relatively very large volume; this has in most cases the form shown in Figs. 120 and 121, but in a few cases it was noticeably elongated, as in Figs. 122 and 123. The last figure shows it to have a transverse constriction around the middle; and this case, together with the fact of its great volume, would show it to be equal potentially to at last two chromosomes; for purposes of description we shall call this the "chromosome *x*." The two other chromosomes, which can always be recognized by their relatively

large volume, are those designated by the letter *k* in Figs. 119–123; these two are of approximately equal volume, and each has about half the volume of the chromosome *x*.

There are accordingly present in the spermatogonic monaster thirteen chromatin elements, of which two (probably the smallest) represent the chromatin nucleoli; of the eleven chromosomes, three are much larger than the others, namely, the one marked *x* and the two marked *k* in the Figs. 119–123. In the metakinesis all these elements are halved longitudinally.

In the following synapsis stage we find a small chromatin nucleolus composed of two parts, which in every way is comparable to the bivalent chromatin nucleolus of the growth period of other *Coreidæ;* this is marked *N. 2* in Figs. 124, 129, 130. This chromatin nucleolus is peripheral in position, and only occasionally has a true nucleolus apposed to it (Fig. 130). Generally its two univalent halves are not closely apposed but more or less separated, often widely separated (*N. 2*, Fig. 131), but the two always come close together to form a dumbbell-shaped, bivalent body before the monaster stage of the first maturation division. Certainly its two components must represent the two univalent chromatin nucleoli of the rest stage of the spermatogonia (*N. 2*, Fig. 118).

During the synapsis stage also ten out of the eleven chromosomes derived from the spermatogonium combine to form five bivalent chromosomes, as will be shown in treating of the maturation divisions. The odd one of the eleven chromosomes does not combine with any other during the synapsis stage, and this is the largest of the chromosomes of the spermatogonium, namely, the chromosome *x*. This element has a remarkable history in the growth period. Through the whole growth period it acts like a chromatin nucleolus in preserving a compact form and in continuing to take the saffranine stain with the use of the double stain of Hermann, while the other chromosomes take the violet stain. It will be remembered that this chromosome *x* becomes distinguishable first in the spermatogonic mitoses (Figs. 119–123), while in the preceding spermatogonic rest stage it cannot be distinguished, for then it takes the violet stain like the other chromosomes and takes part in the formation of the nuclear riticulum just as they do; accordingly it can be concluded that it commences to behave differently from the other chromosomes at the beginning of the growth period of the spermatocyte. In the early synapsis (Fig. 124) it has the same general shape as in the spermatogonic monaster stage (compare the element marked *x* in Fig. 124 with the corresponding one in Figs. 120, 121), but it has greatly increased in volume, as a comparison of these figures show, since it will be recalled that the chromosome *x* of the spermatocytes is a half of the chromosome *x* of the spermatogonia. Later in the growth period the chromosome *x* elongates into the form of a bent rod (Figs. 125–130), which usually lies close to the nuclear membrane (in this point also resembling a chromatin nucleolus); throughout the growth period it keeps its com-

pact structure and smooth outline. When it is beginning to elongate a faintly-marked clear line can be seen in its long axis (Figs. 125, 129), and this is evidently a longitudinal split, comparable to that of the bivalent chromosomes; this split cannot be seen in the telophase nor at any period of the maturation divisions. About coincidently appears a transverse split; this may be a simple annular constriction, or a clear connecting bridge of linin (as in Fig. 128). This transverse constriction, pointing to a bipartite nature, would show that the chromosome *x* is bivalent; but it must have been already bivalent in the spermatogonia (where also a transverse constriction can sometimes be seen, Fig. 123), for it does not unite with any other chromosome in the spermatocytes. I can find no other explanation for its occasional bipartite appearance during the synapsis.

In the later period of the synapsis stage, in the telophase, and in the early prophases of the first maturation division the chromosome *x* undergoes considerable changes in form. The slightly bent rod (Figs. 125, 129) of the early synapsis bends at its middle point, where the transverse constriction was apparent, into the form of a U or V (Figs. 126, 127, 130), or even the form of an S (Fig. 127*b*). The end result of these bendings seems always to be a horseshoe-shape (Fig. 130) or a nearly closed ring (Fig. 127*c*). From the early synapsis stage until the beginning of the prophases of the first maturation mitosis a true nucleolus of varying form is attached to the surface of the chromosome *x* (*N*, Figs. 124, 125, 127–130); occasionally this true nucleolus may be separated into two or three parts, all of them attached to the chromosome. In the prophases of the first maturation division the nucleolus becomes detached from the chromosome, rapidly decreases in size, and becomes lost before the nuclear membrane disappears.

In the early prophases of the first maturation division (Figs. 131, 132) are found the following elements: (1) five bivalent chromosomes, of which all five are shown in Fig. 132, only four in Fig. 131 (all these seen on lateral view); all these show at this stage a well-marked longitudinal split (often of circular or oval outline) and a transverse split (which marks the point of union of two univalent chromosomes); the mode of formation of these elements is very similar to that described by Paulmier (1899) for *Anasa*. (2) The bivalent chromatin nucleolus, the two parts of which may be in close contact (*N. 2*, Fig. 132) or may still be widely separated (*N. 2*, Fig. 131). And (3) the large chromosome *x*, the largest of all the elements, which now has decreased somewhat in volume owing to the greater condensation of its substance; seen from the side, it gives the appearance of a thick horseshoe or a nearly closed ring (*x*, Fig. 131). Of the five bivalent chromosomes, one is always much larger than the others (*K. 2*, Figs. 131, 132), and this was evidently formed by the union of the two large chromosomes *K* of the spermatogonic mitoses (Figs. 119–123).

In the later prophases (Figs. 133, 134) the five bivalent chromosomes condense into the form of dumbbells or sometimes of rings, the large chromosome *K. 2* (Fig. 133) passing through these stages more slowly than the others, so that it often retains loose texture and roughened outlines after the four others have become compact with smooth outlines. The bivalent chromatin nucleolus now has its univalent components generally in rather close apposition (*N. 2*, Figs. 133, 134). The chromosome *x* is very compact in structure, and when seen from the side has squarish form (*x*, Fig. 133), an indentation at one end of which marks the point of apposition of the ends of the primitive horseshoe form—which latter in some cases (Fig. 134) may still be seen at this late stage. Usually the chromosome *x* is longer than broad, and the clear line sometimes found in its long axis does not then represent the primitive longitudinal split, of which there seems no trace at this stage, but the space separating the two arms of the horseshoe.

In the monaster stage of the first maturation mitosis (Figs. 135–137) there are accordingly seven elements (in the cell from which Fig. 136 was drawn, one of the bivalent chromosomes lay out of the plane of the section). These are the bivalent chromatin nucleolus (*N. 2*), the smallest of all; the chromosome *x* (*x*), the largest of all; and five bivalent chromosomes, of which one is almost always recognizable by its greater volume (*K. 2*), and this is the bivalent chromosome formed by the synapsis of the two larger chromosomes *K* of the spermatogonia. The five bivalent chromosomes and the chromatin nucleolus become divided transversely in the metakinesis (reduction division). The chromosome *x* (for successive stages in its division, Figs. 136, 138), which has its long axis coinciding with the plane of the equator, becomes divided into two along its median axis. This would appear at first sight to be a longitudinal (equational) division; but it is not, for we have learned that this peculiar chromosome had first the form of a straight rod, which then bent at its middle point into a U or V, then the arms of the U or V laid themselves parallel to and close together, so that a division along the median axis results now in the separation of these arms, and is accordingly a reduction division.

A view of the second spermatocyte, before its chromatin elements have definitely arranged themselves in the plane of the equator of the spindle, shows also seven elements (Fig. 139): one univalent chromatin nucleolus (*N. 2*); one chromosome larger than the others, a half of the original chromosome *x* (*x* ½); and five univalent chromosomes, one (*K*) larger than the others and directly comparable to one of the large chromosomes *K* of the spermatogonia. In the metakinesis following (Fig. 140) the five univalent chromosomes are divided equationally, and the univalent chromatin nucleolus (*N. 2*) is also divided (but in what plane was not determined). The chromosome *x* ½, however, never becomes divided in this mitosis, but passes undivided into one of the daughter cells (Fig.

140); and in the dyaster stage of the second maturation division (Fig. 141) we see in each daughter cell (spermatid) the chromosomes densely apposed, forming together a rounded, irregular mass, and in only one of the two daughter cells the chromosome $x\,\frac{1}{2}$.

The reduction of the number of chromatin elements in *Protenor belfragei* is accordingly as follows: *Spermatogonium*, two univalent chromatin nucleoli, ten univalent chromosomes, one chromosome x; *first spermatocyte*, one bivalent chromatin nucleolus, five bivalent chromosomes, one chromosome x; *second spermatocyte*, one univalent chromatin nucleolus, five univalent chromosomes, one-half chromosome x; *spermatid*, one semivalent chromatin nucleolus, five semivalent chromosomes, and either present or absent one-half chromosome x. This chromosome x is the odd one of the spermatogonia; it does not unite with any other one in the synapsis stage of the spermatocyte, yet since it sometimes appears bipartite in the synapsis and undergoes a transverse division in the first maturation mitosis, it may perhaps be looked upon as bivalent in both spermatogonium and spermatocyte. If this is a correct conclusion, then the uneven number of chromosomes in the spermatogonia would be the result of two univalent ones remaining there united instead of separating—this compound, bivalent one being the chromosome x. This chromosome, as we have seen, behaves in the rest stage of the spermatogonia like the other chromosomes, but in the growth period of the spermatocytes it acts in many ways like a chromatin nucleolus.

LYGÆIDÆ.

23. *Cymus augustatus* Stal.

Six testes of this species were studied.

There was no material at my service fixed with Flemming's or Hermann's fluids, so not being able to use the triple stain of Hermann I was unable to determine the relations of the chromatin nucleoli in the rest stage of the spermatogonia. The preparations also showed no favorable cells for counting the chromosomes in this generation.

In the synapsis stage there is a rather small, dumbbell-shaped, and so probably bivalent, chromatin nucleolus, which becomes spherical in the following (complete) rest stage of the spermatocyte.

There were no pole views of the chromosomal plate of the first maturation division, but two pole views of the succeeding dyaster are here given (Pl. IV, Fig. 143, showing the chromosomes before taking their definite position in the spindle, while in Fig. 144 they occupy this position and are seen from their ends); here can be counted twelve chromosomes and one smaller body (*N. 2*, probably the chromatin nucleolus, very small in Fig. 144). On lateral views of the first maturation monaster (Fig. 142, which, however, shows only nine of the elements), all the chromosomes usually appear dumbbell-

shaped, and so would be bivalent. Sometimes one of them appears rounded or oval instead of dumbbell-shaped; this may be a bivalent one seen obliquely, or it might possibly be a univalent one; the lack of knowledge of the spermatogonic number does not permit us to decide which.

24. *Ichnodemus falicus* Say

Five testes of this species were studied.

Chromatin nucleoli were not determined in the rest stage of the spermatogonia. But they are very probably present there because two small rounded ones can be seen in the late spermatogonic prophases; whether more than two I could not determine. All the testes examined had been fixed with picro-acetic acid, causing such a swelling and consequent juxtaposition of the chromosomes that in only one case were they sufficiently separated to be counted (Pl. IV, Fig. 145), and here fourteen chromatin elements were present. Since in the first maturation spindle there are always seven bivalent chromosomes, I should think that the fourteen elements of Fig. 145 are univalent chromosomes, and that in this monaster the chromatin nucleoli are hidden.

In the monaster stage of the first maturation division are seen on pole views (Figs. 147, 148) always seven larger elements, which on lateral view are found to be all dumbbell-shaped, and so are probably bivalent. All these seven elements are presumably chromosomes corresponding to the fourteen found in the spermatogonia (Fig. 145). Besides these are to be seen at this stage, and also in the preceding prophases (Fig. 146), two or three smaller elements, which are presumably chromatin nucleoli. Generally three of these are found (*N. 2*, Figs. 146, 147), and generally the case is as in Fig. 146, two larger and one smaller. The two larger being generally of approximately equal volume (Figs. 146, 148), it is quite probable that taken together they may represent one bivalent chromatin nucleolus with separated components. The smaller one sometimes appears transversely constricted, as in Fig. 146, so this one may also be bivalent; if this is the case, then there should be four univalent ones in the spermatogonia. But the number of them could not be determined in the spermatogonia, and in the growth period of the spermatocytes the stain was not favorable for showing their relations.

25. *Peliopelta abbreviata* Uhl.

Five testes of this species were studied.

In a pole view of the spermatogonic monaster (Pl. IV, Fig. 149, the only clear case observed) are found sixteen chromatin segments, ten of which are larger and more elongate than the others, while six are rounded and smaller. The two smallest (*N. 2*) are probably chromatin nucleoli, by analogy with other species of the *Lygæidæ*.

In the growth period of the spermatocytes there is one clearly bivalent chromatin nucleolus, which is frequently apposed to the larger true nucleolus.

In the monaster stage of the first maturation division (Fig. 150, pole view) there are eight elements, the smallest of which is the chromatin nucleolus (*N. 2*); on lateral view all these elements appear dumbbell-shaped and hence are bivalent (Fig. 151). Of the seven chromosomes of this stage, two are very small and five much larger, the two small ones in Fig. 151 being designated as *a. 2* and *b. 2*, while in the figure only three of the large ones are shown. Apparently the two small chromosomes of the spermatocyte correspond to the four small chromosomes of the spermatogonium; thus the bivalent chromosomes *a. 2* and *b. 2* of Fig. 151 would correspond respectively to the univalent chromosomes *a* and *b* of Fig. 149, and the five large bivalent chromosomes of the spermatocyte to the ten large univalent ones of the spermatogonium. It is very evident that in the synapsis stage one of the small univalent chromosomes derived from the spermatogonium never unites with one of the large, for the two univalent components of each small bivalent chromosome of the spermatocyte have approximately the same volume. This speaks, of course, very strongly for the maintenance of the individuality of the chromosomes during these generations.

26. *Œdancala dorsalis* Say

Four testes of this species were studied.

In the rest stage of the spermatogonium are present two chromatin nucleoli of rounded form (*N. 2*, Fig. 152, Pl. IV); these are sometimes attached to one another, sometimes to the true nucleolus (*N*).

In the spermatogonic monaster there are thirteen chromatin elements present, exactly this number being found in all of nine clear cases. Two of these, which are rounded and much smaller than the others, are the chromatin nucleoli (*N. 2*, Figs. 153, 154); the remaining eleven elements are relatively large, elongated chromosomes. All these elements are halved in the metakinesis.

In the synapsis stage the two chromatin nucleoli combine to form one bivalent one, and even up to the rest stage of the spermatocyte (Fig. 155) it remains dumbbell-shaped, with a bridge of linin connecting its univalent components; it is attached to the surface of the true nucleolus (*N*, Fig. 155), and the "double nucleolus" so formed usually lies close to the nuclear membrane.

In the first maturation division there are seven chromatin elements (Fig. 156, pole view of monaster stage), of which the smallest, usually centrally placed one is the bivalent chromatin nucleolus (*N. 2*). Of the six chromosomes, five, when seen on lateral view, are dumbbell-shaped, and so bivalent; but the sixth is oval in outline without any

transverse constriction, about the size of a univalent component of one of the bivalent chromosomes. This is the chromosome marked *x* in Fig. 157; in this figure is shown also the chromatin nucleolus (*N. 2*), but only two of the five bivalent chromosomes. This sixth small chromosome is univalent and unipartite, and evidently is the odd, eleventh, chromosome of the spermatogonium, which had no fellow to combine with during the synapsis. It is always recognizable on lateral views (Fig. 157) by its peculiar volume and form, and even on pole views of the chromosomal plane is recognizable by its lesser depth (Fig. 156, *x*).

The first maturation division halves all seven elements (Fig. 158, anaphase), being a transverse (reducing) division of the five bivalent chromosomes and of the chromatin nucleolus, but in what plane the univalent chromosome (*x*) divides could not be determined on account of its nearly spherical form. Apparently also in the second maturation division all the six chromosomes become divided, since in the spermatid six chromatin elements can frequently be counted; but I am not certain that the sixth chromosome does become divided in this mitosis.

The reduction in the number of chromosomes for this species is accordingly: *Spermatogonium*, two univalent chromatin nucleoli, eleven univalent chromosomes; *first spermatocyte*, one bivalent chromatin nucleolus, five bivalent chromosomes, one univalent chromosome; *second spermatocyte*, one univalent chromatin nucleolus, five univalent chromosomes, one semivalent chromosome.

27. *Oncopeltus fasciatus* Dall.

Eight testes of this species were studied.

The rest stage of the spermatogonium (Pl. IV, Fig. 159) shows usually one comparatively large, elongate chromatin nucleolus (*N. 2*), which is generally peripheral in position; this apparently represents two joined end to end, for sometimes two separate ones can be seen.

In the spermatogonic monaster there are sixteen chromatin elements (Fig. 160). Fourteen are chromosomes and two are chromatin nucleoli, as the relations in the spermatocyte mitoses will demonstrate. But it is difficult to determine which two are the chromatin nucleoli, all sixteen elements being of approximately equal size, though, judging by analogy with the other species of the family, they are probably the smallest two (*N. 2*, Fig. 160).

In the synapsis the fourteen chromosomes unite to form seven bivalent ones. But there is never any very close union of the two chromatin nucleoli, and in the rest stage of the spermatocytes the following conditions are found: (1) the chromatin nucleoli apposed to one another and to the true nucleolus (Fig. 161); (2) apposed to one

another, but separated from the nucleolus (Fig. 162); (3) the chromatin nucleoli separate from one another, and only one in contact with the nucleolus (Figs. 163, 165); (4) the chromatin nucleoli separate from one another and from the nucleolus; (5) the chromatin nucleoli separate from one another, but both attached to the nucleolus (Fig. 164). These conditions do not appear to be stages in position, but rather individual variations. Whenever the two chromatin nucleoli are mutually apposed, it is never an intimate apposition—*i. e.*, they never fuse to form one large rounded one such as is the general rule in the *Pentatomidæ*. Through the rest stage of the spermatocytes each chromatin nucleolus remains elongate (they appear round only when seen from the end). Sometimes each shows a trace of a transverse constriction, and in one case (Fig. 165) one of them was separated into two parts.

Through the prophases of the first maturation mitosis the chromatin nucleoli remain separate from one another, and each is elongate in form (Fig. 166, *N. 2*, showing also all seven bivalent chromosomes on lateral view).

Pole views of the monaster stage of the first maturation division show in most cases nine chromatin elements (Fig. 167); the seven larger ones are chromosomes, all bivalent and on lateral view dumbbell-shaped (Fig. 166); the two smaller, centrally placed ones are the two chromatin nucleoli (*N. 2*, Fig. 167). In one case, ten elements were seen on pole view (Fig. 168); this is very unusual, but it may probably be explained by the assumption that there are here two chromatin nucleoli, six bivalent chromosomes, and two univalent chromosomes formed by the precocious separation of the parts of a bivalent chromosome. A lateral view of the spindle is given (Fig. 169), showing the two chromatin nucleoli, but only two of the seven bivalent chromosomes.

In the metaphase of this mitosis all seven chromosomes become transversely divided (reduction division), and each of the chromatin nucleoli becomes also divided in a plane perpendicular to its long axis (compare Figs. 169 and 170, in each of which only two of the seven chromosomes are shown). A pole view of one cell of the dyaster stage following (Fig. 171) shows the two daughter chromatin nucleoli (*N. 2*) and the seven daughter (univalent) chromosomes (the apparent transverse constrictions on them representing the reappearance of the original longitudinal split; compare the left-hand chromosome of Fig. 170).

The behavior of the chromatin nucleoli is thus different from that of the other *Hemiptera* examined in the following regards: (1) their large size in the spermatogonic monaster (Fig. 160), so that they can hardly be distinguished in volume from the chromosomes; (2) the phenomenon that they remain more or less separate from one another during the growth period of the spermatocytes (Figs. 161–165) and prophases of the first maturation division (Fig. 166); (3) the fact that one or both may appear

bipartite in the growth period (Fig. 165); and (4) the fact that they remain separate from one another in the first maturation division, and that each divides transversely (Figs. 167, 169, 170, *N. 2*). The last mentioned point deserves particular consideration, for a transverse division of a chromatin element in the *Hemiptera* always means a reduction division—*i. e.*, a separation of two whole univalent components of one already bivalent element. From these facts we are led to the conclusion that here the chromatin nucleoli are virtually bivalent in the spermatogonia, and that since the spermatogonic division gives a longitudinal half of each of them to the spermatocytes, that each is already bivalent in the spermatocytes—a bivalence then produced before the synapsis stage of the growth period. This conclusion would explain all their peculiarities listed above. In the spermatogonium accordingly there would be virtually four chromatin nucleoli, twice the number found in the other species of the *Lygæidæ* (with possibly the exception of the not fully explained *Ichnodemus falicus*).

CAPSIDÆ.

28. *Leptopterna dolabrata* Linn.

Three testes of this species were studied.

There were no spermatogonia on any preparations (taken from adults in the last instar before copulation).

In the rest stage of the growth period of the spermatocytes are found the following relations for the chromatin nucleoli: There are two chromatin nucleoli, which (1) are attached to one another but separate from the true nucleolus (Plate IV, Fig. 172); (2) they are attached together and to the true nucleolus (Fig. 173); (3) they are separated from one another but both attached to the true nucleolus (Fig. 175), often at opposite poles of the latter (Fig. 174). In almost all cases they are attached to the true nucleolus, so that Fig. 172 represents an unusual case. Each chromatin nucleolus is probably a univalent one, for it never shows a bipartite appearance and is usually rounded, so that in Figs. 172 and 173 the two together would constitute one bivalent chromatin one; but there cannot be certainty on this point until the number in the spermatogonia is determined.

Pole views of the monaster stage of the first maturation division (Fig. 176) show seventeen chromatin elements, one of which, centrally placed, is always much larger than the others. On lateral view all appear dumbbell-shaped and so are probably bivalent. Probably one of these elements represents the bivalent chromatin nucleolus described for the growth period, then the sixteen remaining would be chromosomes.

29. *Calocoris rapidus* Say

Three testes of this species were studied.

The number of chromatin nucleoli in the rest stage of the spermatogonia was not determined.

In the most favorable pole view of a spermatogonic monaster were counted about thirty chromatin elements (Plate IV, Fig. 177), but these elements were densely grouped so that I could not be positive as to the exact number. Since there are in the spermatocytes fourteen bivalent chromosomes, two bivalent chromatin nucleoli and one that is probably univalent, there would be probably in the spermatogonia twenty-eight univalent chromosomes and five univalent chromatin nucleoli, a total of thirty-three elements.

In the telophase and rest stages of the spermatocytes there is a large true nucleolus, which is remarkable in being flattened against the nuclear membrane (*N*, Figs. 178, 179); it appears sickle-shaped on cross section, and has irregularly lobular outlines on surface view. In these stages there are five small chromatin nucleoli (*N. 2*, Fig. 178); one of these is larger than the others and always spherical in form (the larger one of Figs. 178 and 179), and since it never appears bipartite is presumably univalent; it is frequently attached to the true nucleolus. The four other chromatin nucleoli are arranged in two pairs, the two components of a pair being connected by a band of linin (Figs. 178, 180, only one of the pairs shown in Fig. 179). Each one of these four is very small and spherical, and accordingly probably univalent, and each pair would then be bivalent. Thus there would appear to be in the spermatocytes one larger univalent chromatin nucleolus and two bivalent ones, in each of the latter the univalent components being not closely apposed.

Pole views of the monaster stage of the first maturation division show always sixteen chromatin elements (Figs. 185, 186). Three of these are always distinguishable by their much smaller size (*N. 2*, Figs. 185, 186). These three probably represent the three chromatin nucleoli of the preceding growth period; two of them appear dumbbell-shaped on lateral view (Fig. 181, and one of the two is shown in Fig. 184), obviously representing the two bivalent ones of the growth period; while the third one always appears rounded and never dumbbell-shaped (Fig. 181, the lowest of the elements designated *N. 2*), and on lateral view of the spindle lies nearer one pole of the spindle than the other (*N. 2* Fig. 183), this one obviously representing the univalent chromatin nucleolus of the growth period. Figs. 181–184 represent oblique lateral views of the spindles, so that in each case only one spindle pole is shown, such oblique views giving the best views of the chromatin elements. The thirteen remaining larger elements of the first spermatocytes are chromosomes, and lateral views show that twelve of these are dumbbell-shaped and hence probably bivalent; but the thirteenth is quadrivalent, composed of two bivalent (dumbbell-

shaped) placed side by side with their long axes parallel. This quadrivalent chromosome shows its nature plainly on lateral view (*t*, Figs. 182, 183); on pole view it may always be told by its greater volume (*t*, Figs. 185, 186), sometimes even on pole view it appears slightly constricted (Fig. 186, *t*), the constriction then denoting the plane of apposition of the two bivalent chromosomes of which it is composed; it is placed in the spindle so that the tranverse constriction of each of its component chromosomes lies in the plane of the equator (Figs. 182, 183).

There are accordingly in the first spermatocyte two bivalent chromatin nucleoli, one univalent chromatin nucleolus, twelve bivalent chromosomes, and one quadrivalent chromosome, in all sixteen chromatin elements.

In the spindle of the second spermatocyte are found either fifteen chromatin elements (Fig 188) or sixteen (Fig. 187). This disparity in number is produced by the univalent chromatin nucleolus not dividing in the first maturation mitosis but passing undivided into one of the two daughter cells (second spermatocytes), for it will be remembered that in the monaster stage of the first mitosis it always lies a little outside of the plane of the equator, nearer one pole of the spindle than the other (Fig. 183, *N. 2*). The rest of the chromatin elements of the second spermatocyte are univalent halves of those in the first spermatocyte (the first maturation division is a reduction division), namely, halves of the two bivalent chromatin nucleoli, of the twelve bivalent chromosomes, and of the one quadrivalent chromosome. The latter element can be recognized in the second spermatocytes by its greater size (*t*, Figs. 187, 188), and it here consists of two univalent chromosomes placed in apposition; in the spindle of the first maturation mitosis (Figs. 182, 183) it was so placed that each of its bivalent chromosomes underwent a transverse (reduction) division, just as was the case with the other bivalent chromosomes.

30. *Pœcilocapsus lineatus* Fabr.

Six testes of this species were studied.

There were no spermatogonic mitoses on my preparations, and I could not determine the relations of the chromatin nucleoli in the rest stage of the spermatogonia.

In the rest stage of the spermatocytes are found two chromatin nucleoli (*N. 2*, Fig. 189, Pl. IV); in one testis two additional, very minute chromatin nucleoli seemed to be present, but I could not find them on the other preparations. One of the chromatin nucleoli is very large and clearly bilobed (Fig. 189) so that it would seem to be bivalent; the other is considerably smaller and apparently always rounded, so that it may be univalent. These two chromatin nucleoli are sometimes, but not usually, in mutual contact.

Pole views of the monaster stage of the first maturation mitosis show always eighteen chromatin elements (Fig. 190). One of these is much smaller and one much larger than

the others. Probably two of the eighteen elements correspond to the two chromatin nucleoli of the growth period, but I cannot determine which two they are; if this is so, then there would be here sixteen chromosomes (all apparently bivalent judging from their dumbbell-shape on lateral view), and one bivalent and one univalent chromatin nucleolus. But there can be no surety in regard to these valences without a knowledge of the numbers in the spermatogonia.

31. *Pœcilocapsus goniphorus* Say

Four testes of this species were studied.

There were no spermatogonic mitoses on my preparations (all from adult individuals).

In the rest stage of the spermatocytes there are present four bipartite chromatin nucleoli (*N. 2*, Figs. 192–195, Pl. V), in one single case there were five (Fig. 191). The largest of the four is composed generally of two rods placed side by side (this is shown in lateral view in Figs. 191, 194, in end view in Figs. 192, 193, 195); the lateral view of this one (the lower of the large ones of Fig. 191) sometimes shows that each of its component rods may be transversely constricted, which might imply that each of the rods is bivalent and hence that the whole is quadrivalent. In each of the three small bipartite chromatin nucleoli the univalent components may be closely apposed to one another (Figs. 191, 192), or may be more or less widely separated (Figs. 193–195). Of the four chromatin nucleoli the largest and the smallest are generally attached to opposite poles of the true nucleolus (*N. 2*, Figs. 191–194), though the relative positions vary considerably as shown by the figures; and it is the two which are generally not so attached which have their component parts most widely separated.

Thus there are at least four, possibly five, bivalent chromatin nucleoli in the spermatocytes.

Pole views of the monaster stage of the first maturation mitosis show either seventeen chromatin elements of approximately equal volume (Fig. 197), and this was the rule in two of the testes examined; while in a third testis in the majority of cases there was present a smaller element in addition to the seventeen larger ones (*t*, Fig. 196); possibly this small element may always be present, but frequently escape observation by being closely apposed to one of the larger elements. All these elements appear dumbbell-shaped on lateral view (Fig. 198), and so are probably bivalent.

How may of these eighteen elements are chromosomes and how many are chromatin nucleoli I cannot determine, since the number in the spermatogonia was not ascertained. Possibly the small element (*t* of Fig. 196) may represent the largest chromatin nucleolus of the preceding growth period, and the seventeen remaining be chromosomes; or if all four chromatin nucleoli are represented in the first maturation division, then there would remain fourteen chromosomes.

PHYMATIDÆ.

32. *Phymata* sp. (*P. wolffii* Stal.?).

Nine testes of this species were studied.

In the rest stage of the spermatogonium there are two chromatin nucleoli (*N. 2*, Fig. 199, Pl. V), usually unequal in size; frequently one or both of them is in contact with the true nucleolus (*N*).

In the spermatogonic monaster are seen on pole view (Fig. 200) thirty chromatin elements; two of these certainly represent the chromatin nucleoli, but they offer no peculiarities by which they can be distinguished from the twenty-eight small chromosomes.

In the synapsis stage the twenty-eight chromosomes unite to form fourteen bivalent ones, and the two chromatin nucleoli to form one bivalent one. The latter is in the telophase and rest stage of the spermatocyte (Fig. 201) clearly transversely constricted, showing its bipartite nature, and is always apposed to the surface of the larger true nucleolus (Fig. 201, *N*.).

Pole views of the monaster stage of the first maturation division (Fig. 202) always show exactly fifteen chromatin elements, namely, fourteen chromosomes and one chromatin nucleolus. All these elements are found to be dumbbell-shaped on lateral view (Fig. 203, showing eight of them), so that all are bivalent. The chromatin nucleolus cannot be distinguished in size from the chromosomes.

NABIDÆ.

33. *Coriscus ferus* Linn.

Three testes of this species were studied.

The relations of the chromatin nucleoli were not determined in the small nuclei of the spermatogonia, and the spermatogonic mitoses were not favorable for counting the small, rounded chromosomes.

In the growth period of the spermatocytes there is usually one large bivalent chromatin nucleolus, with its component parts in close apposition (*N. 2*, Fig. 205, Pl. V), attached to the true nucleolus (*N*). Sometimes its component parts (which are of equal volume) are separated from one another, and then both may be attached to the same true nucleolus (Fig. 204), or they may be apposed to separate nucleoli, or only one of them may be apposed to a nucleolus (of which there are generally two, sometimes three). Besides the large, bivalent chromatin nucleolus can be seen in most nuclei a much smaller chromatin nucleolus (Fig. 205) which stains like the larger one; it is generally close to the nuclear membrane, but is occasionally apposed to a true nucleolus.

In the first maturation division there are ten chromatin elements (Fig. 206, in which

five are seen on lateral view and five on pole view, all these elements not having taken their definite position in the equator of the spindle). All are bivalent, as is proved by their bipartite appearance on lateral view. One of them is smaller than the others, and may represent the large (bivalent) chromatin nucleolus of the growth period (*N. 2*, Fig. 206), while the nine remaining elements are probably chromosomes; if this interpretation be correct then the small (univalent?) chromatin nucleolus found in the growth period would not be represented in the first maturation division.

REDUVIIDÆ.

34. *Acholla multispinosa* De G.

Eight testes of this species were studied.

The relations of the minute chromatin nucleoli could not be determined in the rest stage of the spermatogonia.

In the most favorable pole view of a spermatogonic monaster (Pl. V, Fig. 207) could be counted thirty-one chromatin elements. The twenty-four larger elements seen here are univalent chromosomes, the seven smallest are chromatin nucleoli (some of them very minute). Of the latter there are six arranged in three pairs and one that is isolated. Now we shall find that in the spermatocytes there are four bivalent chromatin nucleoli (Figs. 208–211), so that in the spermatogonia there should be eight; and accordingly though only seven are to be seen in Fig. 207, we are justified in concluding that an eighth must be present there but hidden from view by one of the chromosomes. Thus there would be in the spermatogonium in all probability twenty-four chromosomes and eight chromatin nucleoli, in all thirty-two elements.

In the synapsis stage of the growth period the twenty-four chromosomes unite to form twelve bivalent ones. The eight chromatin nucleoli likewise combine to form four bivalent ones, which near the close of the growth period (Figs. 208, 209) are seen to be small bodies attached to the surface of the true nucleolus (*N*). Each is dumbbell-shaped, usually with its component univalent parts in close apposition, but occasionally the latter are more or less separated from one another.

Pole views of the monaster stage of the first maturation division (Fig. 210) show sixteen elements, namely, twelve larger, bivalent chromosomes (dumbbell-shaped on lateral view) and four much smaller chromatin nucleoli (*N. 2*). All these elements are halved in the following metakinesis, which is a transverse (reduction) division, and pole views of the daughter cells (second spermatocytes, Fig. 211) show twelve larger, univalent chromosomes and four smaller, univalent chromatin nucleoli (*N. 2*).

35. *Sinea diadema* Fabr.

Two testes of this species were studied.

The relations of the chromatin nucleoli in the rest stage of the spermatogonia could not be determined.

There were on my preparations only a few spermatogonic monaster stages, and none of these were favorable for determining the number of chromosomes; the chromosomes are small, densely grouped, and particularly minute elements among them might be chromatin nucleoli.

In the rest stage of the spermatocyte are present four small chromatin nucleoli (*N. 2*, Figs. 212, 213, Pl. V), all of them attached to the surface of a large true nucleolus (*N*). One is larger than the others, and appearing on lateral view to be always transversely constricted (Fig. 213) is probably bivalent; and as one of the three smaller ones is sometimes found to be bipartite, this too would be bivalent. The two remaining are apparently always spherical and not transversely constricted, so that they would seem to be univalent. If this interpretation is correct, there would be here two bivalent chromatin nucleoli each with its component parts closely apposed, and one bivalent one with its components separated, that is three bivalent ones in all; and in the first maturation division there are three chromatin nucleoli present (Figs. 214, 215), which would corroborate this conclusion. But since it could not be determined how many there are in the spermatogonia, the relations in the spermatocytes cannot be considered positively demonstrated.

Pole views of the monaster stage of the first maturation division (Figs. 214, 215) show always three minute chromatin elements, which are chromatin nucleoli (*N. 2*), and apparently thirteen larger chromosomes. But a careful examination of these larger elements shows that in every case four bivalent chromosomes form together a plurivalent one, all four being closely apposed and with their long axes parallel to one another. This is best seen on lateral view of the spindle (Figs. 217, 218), where the four chromosomes marked *t* are always found to be grouped close together; in Fig. 216 only these four chromosomes with their mantle fibre attachments are seen on lateral view. Of the four chromosomes thus grouped together, the two middle ones stand in the closest apposition (Fig. 216–218); and on pole views these two middle ones may be so closely apposed as to appear as one long one (*t*, Fig. 214), or a slight transverse constriction marks the division line between them (*t*, Fig. 215). It follows accordingly that the three elements seen on pole view and marked *t* are really four bivalent chromosomes, the two central ones being so closely apposed as to appear generally as one long one. A comparison of the elements designated *t* in Figs. 214 and 215 with the corresponding elements in Figs. 216–218 makes this evident. Accordingly there are really fourteen bivalent (dumbbell-shaped)

chromosomes present, four of which are always grouped together to form a particular group.

The metakinesis halves each of the fourteen bivalent chromosomes transversely (reduction division, Figs. 217, 218), but the four which together form the group that has been described divide later than the others, as can be seen from the figures given; and it is at this metaphase that this group of four can be most easily recognized. Such a group of four closely united bivalent chromosomes has not been found by me in any other Hemipteron.

36. *Prionidus cristatus* Linn.

Four testes of this species were studied.

In the rest stage of the spermatogonium there are apparently five chromatin nucleoli (*N. 2*, Figs. 220–222, Pl. V), two of which are generally considerably larger than the others, and some or all of which may be attached to the true nucleolus (*N*, Figs. 221, 222). But it is difficult to be sure of the exact number, for sometimes not more than four can be seen (Fig. 221).

In the spermatogonic monaster on pole views (Figs. 223, 224) are seen twenty-six chromatin elements, three of which (*N. 2*) are always much smaller and (by analogy with the other species of the *Reduviidæ*) would probably represent the three small chromatin nucleoli of the preceding rest stage. Two of the twenty-six chromatin elements are elongate in form and much larger than the others; these may be chromosomes (which would be more probable), or they might represent the large chromatin nucleoli of the rest stage of the spermatogonium. If there are really five chromatin nucleoli in the spermatogonia, then there would be these five elements and twenty-one chromosomes present in the spermatogonic mitosis. In five pole views I could count exactly twenty-six chromatin elements, in two there were either twenty-six or twenty-seven, and in one either twenty-four or twenty-five; but in all these cases three particularly small and two particularly large elements could be distinguished.

In the rest stage of the spermatocyte (Fig. 225) are found four chromatin nucleoli (*N. 2*) attached to the true nucleolus (*N*). One of these is longer than the others and rod-shaped, and may represent the two larger chromatin nucleoli of the spermatogonium joined into one bivalent one; the three smaller ones may appear rounded or slightly elongate, and these may represent the three small chromatin nucleoli of the spermatogonium; occasionally two of the small ones are apposed together.

There were no maturation mitoses on my preparations (from adults taken in the month of September).

37. *Milyas cinctus* Fabr.

In the single testis of this species studied (an individual of the month of September) there were no mitoses.

In the rest stage of the spermatocytes (Figs. 226–228) are found one long, rod-shaped chromatin nucleolus and two or three smaller ones (*N. 2*), all apposed to the true nucleolus (*N*). The long one is certainly bivalent, since it frequently shows a transverse constriction (Fig. 228) or is bent at the middle point (Fig. 227); on account of the length of each of its component parts it might be concluded that each of them is bivalent—*i. e.*, that the whole element is quadrivalent—but neither of the parts appear bipartite, so that this long chromatin nucleolus would more probably be bivalent. In those cases where only two smaller chromatin nucleoli are present (Fig. 228), each of them is clearly transversely constricted (bipartite) and hence bivalent; where three are present we find that one is bipartite and accordingly bivalent (Fig. 227), the other two are spherical and hence univalent. By comparing Figs. 227 and 228 we find that the two spherical chromatin nucleoli of the former would together represent one of the small bivalent chromatin nucleoli of the latter. Thus we may conclude that there are three bivalent chromatin nucleoli present in these spermatocytes, though the two components of one of them may be separated.

LIMNOBATIDÆ.

38. *Limnobates lineata* Say.

Of this small species I was able to procure the testes of only one (adult) individual. The only stages of spermatogenesis present were spermatocytes in the growth period.

In the large spermatocytes in the rest stage (Pl. V, Fig. 219) is found a large chromatin nucleolus (*N. 2*), usually apposed to the true nucleolus (*N*) in such a manner that the chromatin nucleolus touches with one pole the nuclear membrane, with the other the true nucleolus.

HYDROBATIDÆ.

39. *Hygotrechus* sp.

Twelve testes were studied of this species (from the vicinity of Philadelphia).

The relations of the chromatin nucleoli in the rest stage of the spermatogonia could not be determined, since the nuclei are very small at this stage.

The spermatogonic monaster shows on pole view (Pl. V, Fig. 229) exactly twenty chromatin elements, of which the eighteen largest are chromosomes and the two smallest (*N. 2*) probably chromatin nucleoli.

In the synapsis stage of the spermatocyte (Fig. 230) are seen two small chromatin

nucleoli (*N. 2*), which are not closely apposed; the true nucleolus (*N*) is much larger. In the rest stage the chromatin nucleoli are usually widely separated from one another, and on account of their small size are difficult to discover. In the growth period they do not stain bright red with the saffranine-gentian violet stain of Hermann, but deep violet, even on excellently stained preparations. Such a staining reaction as this I have not found for the chromatin nucleoli of other *Hemiptera*, for in all the other forms examined the chromatin nucleoli take the red saffranine stain intensely even while the chromosomes have taken the violet stain (in the rest stage). Perhaps in *Hygotrechus* the chromatin nucleoli differ chemically less from the chromosomes than in the other *Hemiptera*, and undergo in the growth period changes parallel to those of the chromosomes.

Pole views of the monaster stage of the first maturation division (Fig. 231) show always exactly eleven chromatin elements. On account of the number in the spermatogonia (twenty), I would interpret these as nine bivalent chromosomes, and two univalent chromatin nucleoli which are not combined into one bivalent one. This is very probable, since the two univalent chromatin nucleoli are often widely separated in the growth period; and on lateral views of the monaster stage of the first maturation division, there are found two bodies which are spherical and not dumbbell-shaped. On no lateral view of this monaster stage could I see all the nine chromosomes clearly; but in one case I saw eight of them, all clearly dumbbell-shaped (bipartite), so that probably all nine are bivalent. The first maturation division is reductional, as in the other *Hemiptera*.

40. *Limnotrechus marginatus* Say

Two testes of this species were studied.

There were no spermatogonic monaster stages present, and I could not determine the relations of the chromatin nucleoli in the rest stage of these cells.

In the rest stage of the spermatocyte (Pl. V, Fig. 232) is found a large true nucleolus (*N*), and separated from it, usually close to the nuclear membrane, a rounded chromatin nucleolus (*N. 2*).

Pole views of the first maturation monaster (Fig. 233) show eleven chromatin elements; sometimes one appears much smaller than the others, and it may represent the chromatin nucleolus.

NAUCORIDÆ.

41. *Pelocoris femorata* Pal. Beauv.

Fourteen testes of this species were studied from adult and half-grown individuals of different seasons of the year. There were an abundance of rest stages of spermatogonia and of spermatocytes in the growth period, but no maturation mitoses present.

The chromosomes were counted in a number of pole views of the monaster stage of the spermatogonia, but in most cases they were so densely grouped as to make the numbers obtained very uncertain. In the most favorable case (Pl. V, Fig. 234) apparently twenty chromatin elements are present, but I could not be certain of this number.

The relations of the chromatin nucleoli in the growth period are very puzzling. On preparations stained with Hermann's saffranine-gentian violet, there appear to be a variable number of rounded bodies of different volume which stain bright red; sometimes they are arranged in pairs, sometimes in long chains, sometimes they show no regular arrangement whatsoever. One nuclear body, much larger than the others and generally irregular in outline, may be a true nucleolus. If all the red-staining bodies are chromatin nucleoli, they would seem to be present in an unusually large number in this species. A study of further material will be necessary to explain the nature and relations of these bodies.

BELOSTOMATIDÆ.

42. *Zaitha* sp.

There are two species of this genus known in the vicinity of Philadelphia where I collected my material, namely, *Z. fluminea* Say and *Z. aurantiacum* (Leidy), but which species it was that I collected I omitted at the time to determine. Ten testes were examined.*

In the rest stage of the spermatogonium (Fig. 235) are present two small chromatin nucleoli (*N. 2*) apposed to a large true nucleolus (*N*).

Pole views of the spermatogonic monaster stage (Figs. 236, 237) show twenty-four chromatin elements, of which the smallest two (*N. 2*) represent the chromatin nucleoli, and the remaining twenty-two are chromosomes. Of the chromosomes, four are always elongate and much larger than the others (*t*, Figs. 236, 237).

In the synapsis stage of the growth period the twenty-two chromosomes unite to form eleven bivalent ones. In the rest stage of the spermatocytes there are two univalent chromatin nucleoli, sometimes joined to make a bivalent one, attached to the surface of the true nucleolus.

Pole views of the monaster stage of the first maturation division (Fig. 238) show

* In my "Note on the Genital Organs of Zaitha" (*American Naturalist*, Vol. xxxiv, 1900). I described the structure of these testes. To the text figure B given in that paper, I would add now that the earlier stages of spermatogenesis are to be found in what I called the "terminal fibres" at the proximal end of the testis, these fibres being five long and much convoluted slender tubes which interlace together and form a rounded whitish mass at the extreme proximal end of the testis; they were not correctly represented in the figure cited. In adult individuals it is only in this portion of the testis that the earlier spermatogenetic stages occur, all the rest of the testis being filled with spermatozoa. It is necessary to collect individuals in the month of May (shortly before the copulation), in order to obtain the stages of the maturation divisions.

always thirteen chromatin elements. Two of these are much smaller than the others (*N. 2*), and are obviously the univalent chromatin nucleoli, which at this stage do not make up a bivalent one. The eleven large elements are chromosomes, and since all of them appear dumbbell-shaped on lateral view, they are all bivalent. Two of the eleven chromosomes have a markedly greater volume than the others (*t 2*, Fig. 238), and these correspond to the four large chromosomes of the spermatogonia (*t*, Figs. 236, 237); that is to say, in the synapsis the four large chromosomes derived from the spermatogonia unite together to form two bivalent ones, and a large one never appears to unite with a small one.

The first maturation mitosis is a reduction division, and each daughter cell (second spermatocyte) receives eleven whole univalent chromosomes.

III. General Conclusions.

1. The process of spermatogenesis in the Hemiptera, and the individuality of the chromosomes.

In the *Hemiptera heteroptera* we find, as generally elsewhere in the *Metazoa*, a number of generations of spermatogonia in each of which all the chromatin elements are halved in metakinesis (apparently in all cases equationally), the last generation producing spermatocytes of the first order. These spermatocytes enter upon a growth period of long duration, which is followed by the first maturation division, resulting in the formation of spermatocytes of the second order; and in the second maturation division the spermatocytes of the second order are divided into spermatids. There are always exactly two maturation divisions and no more. The metamorphosis of the spermatids into the spermatozoa has not been studied by me, and has not the broad comparative interest of the preceding stages, but it has been described by Henking (1890) and Paulmier (1899).

The growth period of the spermatocytes is of the greatest interest, for here are a remarkable series of changes not found in any other generation of the germ cells nor, so far as is known, in any somatic cells. And the most important of these changes are found in the synapsis stage of the growth period. The synapsis stage is well-marked in all the *Hemiptera* examined by me without exception, characterized by a dense grouping of the chromatin loops; the citations given by me in my study on *Peripatus* (1901) show that it seems to be present in almost all, if not all the *Metazoa* in which the spermatogenesis and ovogenesis has been carefully examined. The dense grouping of the chromosomes in this stage is not an artifact produced by faulty fixation methods, as McClung (1900) has recently maintained, for exactly the same appearances are to be

found after the action of most diverse fixatives. The dense and interlacing grouping of the chromosomes that is so characteristic for the synapsis of Insects, Copepods, Ascaris and some other forms, is, however, not found in all; thus it does not appear to occur in *Salamandra* (Meves, 1896).

Moore (1895) first gave the name "synaptic phase" to that stage in the growth period of Elasmobranchs when the reduction in the number of the chromosomes takes place. Accordingly, the criterion of the synapsis stage is first of all the combination of univalent chromosomes to form bivalent ones; whether the chromosomes are then densely grouped or not is of secondary importance. A special chapter of the present paper is given to the broader significance of this stage.

In all the *Hemiptera* examined, and also in *Peripatus*, I have found that bivalent chromosomes are formed in the synapsis by a union, end to end, of every two univalent chromosomes. All other writers on this stage, with the exception of Brauer, have been unable to determine how the univalent chromosomes become united together; Brauer's (1893 b) careful study of the growth period in the spermatogenesis of *Ascaris* rendered it very probable that it is a union end to end, but his figures do not prove it absolutely. Certain writers state that the reduction of the number in chromosomes is effected by the chromatin spirem segmenting into only half the normal number of chromosomes. This is, however, an incorrect statement, inasmuch as the reduction in number is occasioned in some cases (*Hemiptera*, *Peripatus*) before the "spirem" stage of the first maturation division, and inasmuch as in most cases, if not all, there is no continuous *chromatin* spirem found at any time during the growth period and prophases of the first maturation mitosis.

Accordingly in the *Hemiptera* the reduction in number of the chromosomes is effected in the synapsis stage, a long while before the maturation divisions, by a union end to end of every two chromosomes. During the synapsis stage the chromosomes become split longitudinally, as was first shown by Paulmier (1898, 1899) for *Anasa*—a process that I had overlooked in my former paper (1898). Each bivalent chromosome is thus both transversely and longitudinally split before the maturation mitoses, the transverse split represented by the band of linin joining the approximated ends of the two univalent chromosomes.

At the close of the growth period there is a well-defined rest stage in most *Hemiptera*, when chromosomal boundaries are practically indistinguishable; but in the *Coreidæ* and *Reduviidæ* there appears to be no such stage, and accordingly such a stage would appear to have no broad significance.

In the early prophases of the maturation divisions the chromosomes are bivalent but quadripartite, each one being transversely and longitudinally split; in the later pro-

phases, up to the monaster stage of the first mitosis, the longitudinal split generally closes temporarily. The definitive form of these chromosomes in all the *Hemiptera* examined is that of a dumbbell which may be either straight or bent; ring forms are more infrequent, but are occasionally found in all species. In the *Hemiptera*, as in *Peripatus*, each ring may be conceived as a dumbbell which has become bent until its ends meet, and accordingly the hollow of the ring is not the longitudinal split, but a space separating the univalent chromosomes. That is to say, generally only one end of one univalent chromosome is joined to one end of the other, but in the ring form both ends of the one are joined with both ends of the other. In all bivalent chromosomes that have not the ring form a longitudinal axis can be plainly determined, and generally each univalent chromosome is elongated in the same line; the constriction perpendicular to this long axis is a true transverse division, and is the band of linin joining the ends of the two univalent chromosomes. This orientation of the axes of the bivalent chromosomes allows the positive determination of the manner in which the chromosomes are halved in the maturation divisions.

In the first maturation division in all the *Hemiptera* examined the bivalent chromosomes are transversely divided, *i. e.*, whole univalent chromosomes are separated; in the anaphase of this division the longitudinal split of the univalent chromosomes reappears, and in the second maturation mitosis the univalent chromosomes are halved through the plane of this split (equation division). The valences of the chromosomes in the successive generations are accordingly: *spermatogonium*, univalent; *first spermatocyte*, bivalent; *second spermatocyte*, univalent; *spermatid*, semivalent. The classing of the chromosomes as semivalent in the spermatid may appear surprising, for they have always been considered univalent; but they must be considered semivalent with reference to the number in the spermatogonia in those cases, as in all the *Hemiptera* examined, where the second maturation division follows immediately upon the first without any indication of an intermediate rest stage.

Thus in the *Hemiptera*, as in *Peripatus*, the maturation divisions do not accomplish the reduction in number of the chromosomes, for this takes place long before in the growth period; the first maturation division separates entire univalent chromosomes (*pseudoreduction*, Rückert, 1894), the second halves each univalent chromosome equationally, and thus halves the chromatin mass. Though the chromosomes of the spermatid are logically semivalent with reference to those in the spermatogonia, yet they are potentially univalent on account of the increase in mass of the chromatin during the growth period (where at least a doubling of the mass occurs).

In my study on *Peripatus* (1901) it was shown that the individuality of the chromosomes is maintained from the last spermatogonic mitosis up to and through the maturation

divisions; and in that paper I have referred to the observations of other workers corroborative of the individuality of the chromosomes, so that they need not be recalled here. In such *Hemiptera* as the *Coreidæ* there is no true rest stage in the growth period, so that definite chromosomal outlines can be determined throughout the growth period. And even in those *Hemiptera* where a rest stage does occur in this period, in which the chromosomes become reticular and practically indistinguishable from one another, all evidence renders it probable that the chromosomal individuality is retained through this period—*i. e.*, that a particular univalent chromosome of the maturation mitosis represents a particular one of the spermatogonia. This evidence is as follows: The chromatin nucleoli, which are only modified chromosomes, retain their compact form and so are readily distinguishable throughout all periods of these generations. Then in those cases where there is an uneven number of chromosomes in the spermatogonia, and the odd one remains univalent in the spermatocytes, this odd one can always be distinguished in the maturation mitoses. Then where two of the spermatogonic chromosomes are particularly large, there is always found in the first maturation mitosis one particularly large bivalent chromosome which can only correspond to those two. Further, after a rest stage there is always the same number of chromosomes as were present before that rest stage. All this evidence speaks for a chromosome of a spermatogonium corresponding to a chromosome of a spermatocyte; and if in these generations there is a maintenance of chromosomal individuality, there is a probability that there is such a maintenance through all generations of the germinal cycle. But this conclusion by no means implies that a chromosome of one generation is actually the same as a chromosome of another. For we know that each chromosome is halved in an equation division, and that each daughter chromosome so produced must increase to a volume equal to that of the mother chromosome before it enters upon a second mitosis. Thus new substance must continually be elaborated by the chromosomes during the rest stages, and in the course of this elaboration the old substance of the chromosome and its physical form are correspondingly changed. There is no evidence that chromosomal substance remains unchanged from generation to generation, for all evidence shows that it undergoes metabolic change and growth in the rest stages. But nevertheless it seems very probable that a chromosome of one generation is a derivative of a particular chromosome of the preceding generation, and that the chromosomes may thus be said to maintain themselves as entities through successive generations.

There are many cases where chromosomal boundaries are indistinguishable in the rest stages, so that in such cases it has been argued that the chromosomes show no individuality. But these are negative examples, and positive cases that speak for the maintenance of the chromosomal individuality must be considered as the decisive ones. No

other assumption can so well explain the maintenance of a constant number. The fact that the chromosomes build up new substance at one stage, and give off waste products at another, does not invalidate our conclusion. Those who deny the maintenance of chromosomal individuality, on the basis of a study of objects where the chromosomes do not appear to be continuous from generation to generation, are not justified in concluding that there is never a maintenance of the individuality until they have examined the positive cases. And since there have been shown to be positive cases, we must conclude (1) either that in all normal mitoses of the germinal cycle the chromosomal individuality is maintained, or (2) that it is preserved in some cases but not in others. The fact that it has been demonstrated for some cases renders it probable that is maintained in all cases, even though it cannot be demonstrated in all.

2. *The chromatin nucleoli.*

The term "chromatin nucleolus" was applied by me (1898) to the remarkable chromatin element, in form like a nucleolus but in behavior and staining reactions like a chromosome, in the nuclei of spermatocytes of *Euchistus* (*Pentatoma*); the name was given in order to denote this double similarity, though I fully realized that this structure was in all essentials a modified chromosome. A special monograph (1899 b) was devoted by me to the true nucleolus (plasmosome, Ogata) of Metazoan cells; and in a lecture at the Woods Holl laboratory (1898 b) I classified the other "nucleolar" structures as "karyosomes, which are merely thickened nodal points of the chromatin reticulum; further, what I shall term the 'chromatin-nucleus' [typographical error for 'chromatin nucleolus'], which is found in certain spermatocytes; and then various structures which stain neither like the true nucleolus nor the chromatin, and to which such terms as 'Paranucleoli,' 'Nebennucleoli,' and 'Pseudonucleoli' have been applied. It is one of the most difficult questions to determine the nature and correspondence of the latter structures. . . . . But from the cases studied by me, it would appear that some of these structures in *Metazoa* probably must be placed within the category of true nucleoli, and be regarded as true nucleoli of a different chemical nature; . . . . our criterion of nucleoli probably should not be based as much upon chemical as morphological facts."

The chromatin nucleolus is a modified chromosome, as both my earlier and the present observations show. The term may have been injudiciously chosen, since I myself showed that it has nothing in common with a true nucleolus except sometimes in the form and in containing vacuoles. In its stead McClung (1899) has given the name "accessory chromosome," and Paulmier (1899) the name "small chromosome." But McClung's term is not satisfactory in not being at all definite, and Paulmier's term is not

applicable to those cases where it is as large as the other chromosomes. Hence I consider that confusion in terminology would be best avoided by retaining my original term, "chromatin nucleolus."

In my first description of the spermatogenesis of *Euchistus* (1898 a), I stated that I could not find chromatin nucleoli in spermatogonia, and that one appeared for the first time in the spermatocyte by a metamorphosis of one of the fourteen chromosomes; this was an error that I have corrected in the present paper, for in *Euchistus*, just as Paulmier (1899) correctly described for *Anasa*, there are two chromatin nucleoli in the spermatogonium, and these unite in the spermatocyte to form one bivalent one. And in all the *Hemiptera* examined by me the larger chromatin nucleoli of the spermatocytes are derivatives of chromatin nucleoli of the spermatogonia, except the remarkable "chromosome x" of *Protenor*, to which we shall return. As far as I have been able to determine, the chromatin nucleoli are always halved in the mitoses of the spermatogonia.

(a) *General Characteristics.*

The chromatin nucleoli are morphologically chromosomes, undergoing division in mitosis like the other chromosomes, but differing from them in the rest stage by preserving a definite (usually rounded) form. There is also another difference which is of great use in their study: by the use of the double stain of Hermann, saffranine and gentian violet, the chromosomes proper stain red only in mitosis and violet in the rest stage, while the chromatin nucloli stain red in the rest stage also, and so can be sharply distinguished from the chromatin of the chromosomes.* Thus the chromatin nucleoli of the *Hemiptera* seem to retain at all stages the stain characteristic for the substance of the chromosomes when in the height of mitosis. In the *Hemiptera* examined the true nucleoli never takes this red stain, but take the violet, so that they may in this way be easily distinguished from the chromatin nucleoli.† With iron-hæmatoxylin staining the chromatin nucleoli stain more intensely than the chromosomes in the rest stage; but with this stain the true nucleoli generally stain deep black like the chromatin nucleoli, so that it is far less satisfactory than the preceding method for differentiating these two structures. With the triple stain of Ehrlich-Biondi-Heidenhain, the chromatin nucleoli

* The only exception to this staining reaction was found in the spermatocytes of *Hygotrechus*, where in the rest stage the chromatin nucleoli always take the violet stain.

† However, in cells of many other *Metazoa* I have found that the true nucleoli show a particular electivity for the saffranine, so that chemical reactions are not tests for true nucleoli; nucleoli may differ chemically from one another, even in the same cell at the same stage, or at different stages, and no better case of this may be mentioned than the ovocytes of the growth period of *Gryllus*. In the *Hemiptera* the true nucleoli are generally much larger than the chromatin nucleoli, more or less irregular in outline, and they usually occupy a more or less central position in the nucleus (though I have mentioned two or three exceptions), while the chromatin nucleoli in the spermatocytes are generally in contact with the nuclear membrane.

and chromosomes in the rest stage are green, the true nucleoli red; with Delafield's hæmatoxylin and eosin, the chromatin nucleoli and chromosomes stain blue, the true nucleolus red; with both these methods the chromatin nucleoli stain more intensely than the chromosomes in the rest stage.

In the spermatogonia the chromatin nucleoli are generally small, often very minute and difficult to distinguish. They are apparently always halved in the spermatogonic mitoses, though I have not been able to determine this for all species. They always increase in volume during the growth period of the spermatocytes; the relative amount of this increase varies in different species, but it is a number of times greater than in the spermatogonia. During this increase in mass there can be found in most cases a clear vacuole in the chromatin nucleolus, so that the increase would appear to be not so much one of the proper substance of the chromatin nucleolus as of an intussusception of fluid from without; I have not figured these vacuoles in the present paper, but showed them in a preceding one (1898 a). In the prophases of the first maturation division the chromatin nucleolus decreases very considerably in volume, until when the monaster stage is reached its volume may be very little greater than in the spermatogonia. It is possible that its increase in mass during the growth period may be due in part to a decondensation of its substance, but in some part at least it is due to the above-described taking in of fluid substance from without.

In the spermatogonia they are irregular in position, sometimes close together, sometimes separated, but usually not in contact with the nuclear membrane; in these cells they are very frequently in all species apposed to the true nucleoli.* In the growth period of the spermatocytes they are more regular in position: thus they are separated from the true nucleoli and then always in contact with the nuclear membrane in *Euchistus*, *Mormidia*, *Peribalus*, *Cosmopepla*, *Nezara*, *Brochymena*, *Trichopepla*, *Eurygaster*, *Metapodius*, *Anasa*, *Chariesterus* (generally), *Corizus*, *Harmostes*, *Calocoris*, *Hygotrechus*, *Limnotrechus*; they are, as a rule, apposed to the true nucleoli in *Podisus*, *Perillus*, *Cœnus*, *Alydus*, *Protenor*, *Peliopelta* (not always), *Œdancala*, *Oncopeltus* (not always), *Leptopterna*, *Pœcilocapsus*, *Phymata*, *Coriseus*, the *Reduviidæ*, *Limnobates* and *Zaitha*. We shall refer again to the significance of this apposition. A chromatin nucleolus and a true nucleolus closely attached together constitute a "double nucleolus;" it remains to be shown whether the "double nucleoli" of certain cells of other *Metazoa*, as *e. g.* in the cells of Sertoli of *Salamandra* and *Mus*, as described by Hermann and others, may also be cases of apposed chromatin nucleoli and true nucleoli. It is very characteristic of the chromatin nucleoli of the spermatocytes in the growth period to be closely apposed to the nuclear membrane. And when they are apposed to true nucleoli,

* In the spermatogonia there are in all species an irregular number of true nucleoli, but in the spermatocytes of most of the species examined there is regularly one large one.

they retain this position if they are of comparatively large size (as in most *Pentatomidæ*) but do not when they are of comparatively small size (as particularly in the *Reduviidæ*). Now the true nucleolus is rarely in contact with the nuclear membrane when it is not apposed to a chromatin nucleolus, so that when the two are mutually apposed it appears to depend upon their relative volumes whether they will be peripheral or central in position—a very large chromatin nucleolus pulling to the periphery the true nucleolus, a small chromatin nucleolus being pulled by the true nucleolus toward the centre of the nucleus.

That the chromatin nucleoli are morphologically chromosomes is shown particularly in mitosis, when they simulate in form and divide like chromosomes; an examination of the chromatin nucleoli, designated *N. 2*, in the plates of the present paper, demonstrate this point. The univalent chromatin nucleoli of the spermatogonia generally unite to form bivalent ones in the synapsis stage just as do the chromosomes, and generally the number of them in the spermatocytes is just half of that in the spermatogonia (certain exceptions shall be considered later). In the first maturation division each bivalent chromatin nucleolus is halved transversely (reduction division) just as are the bivalent chromosomes.

Thus the chromatin nucleoli are essentially chromosomes, but chromosomes which preserve a compact form and dense structure throughout the rest period.

(b) *Number and Valence.*

It is most frequently the case that there are two univalent chromatin nucleoli in the spermatogonia, as in the *Pentatomidæ, Eurygaster, Coreidæ, Peliopelta, Œdancala, Phymata, Coriscus* (?), *Hygotrechus, Zaitha*. In these cases the two chromatin nucleoli join together to form one bivalent one in the growth period; but in *Euchistus tristigmus* and *Chariesterus* the two frequently remain separate in the growth period, yet this is not a complete separation for there would seem to be a linin connection between the two, since they generally become more closely approximated at the time of the first maturation division when such a linin connection may be seen. But in some of these species (*Peribalus, Cænus, Trichopepla, Coriscus*) besides the bivalent one are found one or two (three or four in *Trichopepla*) much smaller ones, which appear to be univalent, in the spermatocytes; whether these are represented in the spermatogonia or whether they arise in the spermatocytes for the first time, I could not determine on account of their minuteness.

In *Cymus* and *Ichnodemus* I could not determine the number in the spermatogonia, but since here there is one bivalent one in the spermatocytes, there would probably be two univalent ones in the spermatogonia. The same would probably be true for *Corizus* and *Leptopterna*.

The *Reduviidæ* and certain *Capsidæ* show a larger number of chromatin nucleoli.

Thus in *Acholla* there are eight in the spermatogonia which form four bivalent ones in the spermatocytes; in *Sinea* and *Milyas* there are three bivalent ones in the spermatocytes, and accordingly probably six in the spermatogonia; in *Prionidus* there are apparently five in the spermatogonia, two of which unite in the spermatocytes to make one bivalent one while the three others remain univalent. In *Calocoris* there are in the spermatocytes two bivalent and one univalent; in *Pœcilocapsus lineatus*, one bivalent and one apparently univalent; in *P. goniphorus* certainly four, and possibly five, bivalent chromatin nucleoli in the spermatocytes. In these forms one or more of the chromatin nucleoli may have their component parts more or less widely separated, but these separated components generally come together before the first maturation mitosis.

Bivalent chromatin nucleoli which have their components in close mutual apposition appear always to be transversely divided in the first maturation division (reduction division); for the behavior of those which are univalent I refer to the chapter on "Observations."

Oncopeltus affords the interesting case where the two chromatin nucleoli of the spermatogonia are apparently each bivalent—bivalent elements in a generation where all the chromatin elements are usually supposed to be univalent.

(c) *The Peculiar Chromosome of Protenor.*

In *Protenor*—for the details compare the description in the chapter on "Observations"—there are two chromatin nucleoli in the spermatogonia, which in the spermatocytes unite to form a bivalent one, as we have just seen to be quite generally the rule in *Hemiptera*. But the largest of the chromosomes of the spermatogonic mitoses, the "chromosome x," does not behave in the growth period of the spermotocytes like the other chromosomes, but is similar to a chromatin nucleolus in preserving a compact form and in retaining the saffranine stain. This is the odd chromosome, the eleventh, which does not combine with any other during the synapsis stage, and which cannot be distinguished in the rest stage of the spermatogonia because there it behaves exactly like the other chromosomes, and takes part in the formation of the nuclear reticulum. This is the only case in the *Hemiptera* where one chromosome becomes differentiated into a chromatin nucleolus for the first time in the spermatocyte generation, unless the minute chromatin nucleoli of *Peribalus*, *Cœnus*, *Trichopepla* and *Coriscus* may be found to have a similar history.

(d) *Function.*

All the observations show that the chromatin nucleoli are modified chromosomes, which behave essentially like the chromosomes in mitosis but quite differently in the rest stage.

Paulmier (1899) has suggested that they are degenerate chromosomes. This would

seem to be true to some extent, but not wholly correct for the following reasons. In a large number of the species examined the chromatin nucleoli are regularly closely apposed to the surfaces of true nucleoli. Now it seems probable that the true nucleoli are masses of substances formed by the metabolism of the cell, probably waste substances (Montgomery, 1899 b). When we find accordingly the mutual apposition of them to chromatin nucleoli, it would be permissible to conclude that the chromatin nucleoli are chromosomes which are especially concerned with nucleolar metabolism. And this I think would be the correct interpretation. The chromatin nucleoli are in that sense degenerate, that they no longer behave like the other chromosomes in the rest stages; but they would appear to be specialized for a metabolic function. Thus it might be that in the Insects the chromatin nucleoli are those chromosomes which either exert a greater metabolic activity than the other chromosomes, or which carry out some special kind of metabolism; and from this point of view they would certainly seem to be much more than degenerate organs. As to their origin, compare the chapter on the "Number of Chromosomes."

Like the chromosomes, the number of chromatin nucleoli in the germ cells appears to be a fixed one for the species. In somatic cells they are often more numerous than in the germ cells, however, but that somatic difference will not be discussed in this paper which concerns itself with the germinal cycle.

(e) *Occurrence.*

Chromatin nucleoli were found by me in all the *Hemiptera* examined, and I have found them also in *Coleoptera* (*Harpalus*) and *Orthoptera* (*Gryllus, Ceutophilus*). McClung (1899, 1900) has described them in various *Orthoptera*, in some forms of which they are larger than the chromosomes. Finally, my student, Miss Wallace (1900), has found them in the spermatogenesis of a spider (Agalenid).

Accordingly they would seem to be present in the Insects and Arachnids, but are apparently absent in the *Crustacea*, and I have shown (1901) that they are not present in *Peripatus*.

It is, however, quite possible that they will be discovered in other forms, if proper attention is paid to them. The question of their ontogenetic origin will be considered later (compare the section on the "Significance of the uneven number" of chromosomes).

3. *The Number of Chromosomes.*

The following table shows the number and valence of the chromosomes and chromatin nucleoli in the *Hemiptera* examined, all of which has been explained in detail in the chapter on "Observations." The abbreviation "*univ.*" has been employed for "univalent," and "*biv.*" for "bivalent."

Species.	Spermatogonia.				Spermatocytes.			
	Chromosomes.		Chromatin nucleoli.		Chromosomes.		Chromatin nucleoli.	
	No.	Valence.	No.	Valence.	No.	Valence.	No.	Valence.
Euchistus variolarius	14	univ.	2	univ.	7	biv.	1	biv.
E. tristigmus	12	univ.	2	univ.	6	biv.	1 or 2	biv. or univ.
Podisus spinosus	14	univ.	2	univ.	7	biv.	1	biv.
Mormidea lugens	14	univ.	2	univ.	7	biv.	1	biv.
Peribalus limbolaris	14	univ.	2	univ	7	biv.	1	biv.
Cosmopepla carnifex	16	univ.	2	univ.	8	biv.	1	biv.
Nezara hilaris	14	univ.	2	univ.	.....	.....	.....	.....
Brochymena sp.	14	univ.	2	univ.	7	biv.	1	biv.
Perillus confluens	14	univ.	2	univ.	7	biv.	1	biv.
Cœnus delius	12	univ.	2	univ	6	biv.	2	1 biv. 1 univ.
Trichopepla semivittata	14	univ.	2	univ.	7	biv.	4 or 5	1 biv. 3 or 4 univ.
Eurygaster alternatus	.....	.....	.....	.....	6	biv.	1	biv.
Anasa tristis	20	univ.	2	univ.	10	biv.	1	biv.
A. armigera	20	univ.	2	univ.	10	biv.	1	biv.
A. sp	20	univ.	2	univ.	10	biv.	1	biv.
Metapodius terminalis	20	univ.	2	univ.	10	biv.	1	biv.
Chariesterus antennator	.....	.....	.....	.....	12	biv.	1	biv.
Alydus pilosulus	12	univ.	2	univ.	6	biv.	1	biv.
A. eurinus	11	univ.	2	univ	6	5 biv. 1 univ.	1	biv.
Corizus lateralis	.....	.....	.....	.....	6	biv.	2 or 3	1 biv. 1 or 2 univ.
Harmostes reflexulus	11	univ.	2	univ.	6	5 biv. 1 univ.	1	biv.
Protenor belfragei	11	10 univ. 1 biv.	2	univ.	5	biv.	2	biv.
Cymus angustatus	.....	.....	.....	.....	12	biv. (all?)	1	biv.
Ichnodemus falicus	14	univ.	2	univ.	7	biv.	2 or 3	?
Peliopelta abbreviata	14	univ.	2	univ.	7	biv.	1	biv.
Œdancala dorsalis	11	univ.	2	univ.	6	5 biv. 1 univ.	1	biv.
Oncopeltus fasciatus	14	univ.	2	biv.	7	biv.	2	biv.
Leptopterna dolabrata	.....	.....	.....	.....	16	biv.	1	biv.
Calocoris rapidus	28?	univ.	5?	univ.	14	biv.	3	2 biv. 1 univ.
Pœcilocapsus lineatus	.....	.....	.....	.....	16?	biv.	2	1 biv. 1 univ.
P. goniphorus	.....	.....	.....	.....	17? 14?	biv.	4	biv.
Phymata sp.	28	univ.	2	univ.	14	biv.	1	biv.
Coriscus ferus	.....	.....	.....	.....	9	biv.	2	1 biv. 2 univ.
Acholla multispinosa	24	univ.	8	univ.	12	biv.	4	biv.
Sinea diadema	.....	.....	.....	.....	14	biv.	3	biv.

Species.	Spermatogonia.				Spermatocytes.			
	Chromosomes.		Chromatin nucleoli.		Chromosomes.		Chromatin nucleoli.	
	No.	Valence.	No.	Valence.	No.	Valence.	No.	Valence.
Prionidus cristatus?.....	21?	?	5?	univ.	.....	.....	.....	.....
Milyas cinctus.....	.....	.....	.....	.....	.....	.....	3	biv.
Limnobates lineata.....	.....	.....	.....	.....	.....	.....	1	biv.
Hygotrechus sp.....	18	univ.	2	univ.	9	biv.	2	univ.
Limnotrechus marginatus.....	.....	.....	.....	.....	10?	biv.	1?	biv.?
Pelocoris femorata.....	20?	.....	.....	.....	.....	.....	.....	.....
Zaitha sp.....	22	univ.	2	univ.	11	biv.	2	univ.

The following general deductions may be drawn from the consideration of these facts. Whenever there is an even number of univalent chromosomes in the spermatogonia, they unite in the synapsis to produce exactly half this number of bivalent chromosomes. When there is an uneven number in the spermatogonia (*Alydus eurinus, Harmostes, Protenor, Œdancala*) all but one of the chromosomes unite in the synapsis to form bivalent chromosomes, while the odd one remains single.

(a) *Number and Genetic Relationship.*

At the outset of the present study I was particularly interested to determine whether the numbers of chromosomes might afford clues to the relationship of the group of the *Hemiptera heteroptera;* that is, to learn, if possible, whether the number would afford a taxonomic criterion. Most of the families of the *Hemiptera* are very rich in species, however, and I have been able to procure only a few species for study, so that the present beginning must be continued on many more species before any conclusion can be reached.

In the *Pentatomidæ*, counting the chromosomes in the spermatogonia, and not including the chromatin nucleoli, we find the number varies between twelve and sixteen, fourteen being most usual; in the *Coreidæ* we find twenty in *Anasa* and *Metapodius*, in *Alydus* eleven and twelve, in *Harmostes* and *Protenor* eleven. In the *Lygæidæ*, fourteen in *Ichnodemus, Peliopelta* and *Oncopeltus*, eleven in *Œdancala*, and probably twenty-four in *Cymus*. These were the three families of which the most species were examined. From these numbers it will be seen that there is considerable variation in number for the different species of one and the same family. Accordingly we must conclude either (1) that the number of chromosomes is easily modified and changed, so that it has little

taxonomic value; or (2), that the families of the *Hemiptera heteroptera*, as they are at present defined, are artificial and not natural groups. I would incline to the latter view, since all our facts would show that chromosomes are very conservative structures, and that the germinal cycle is conservative; probably the soma may be modified by the action of the environment to considerable extent, before any such action would produce a change in the number of chromosomes. If this standpoint is correct, then the number of the chromosomes would be a very important consideration in deciding the relationship of species; thus the *Coreidæ* would have to be subdivided into a sub-group with twenty to twenty-four chromosomes (*Anasa, Metapodius, Chariesterus*), and into one with eleven or twelve chromosomes (*Alydus, Corizus, Harmostes, Protenor*). But it would be a *reductio ad absurdum* to say that all forms with twelve chromosomes must be related, or all forms with twenty; the relative boundaries of a family must still be determined from the standpoint of broad comparative anatomy, and then within a group so defined the chromosomal number might be used as a basis for further subdivision.

(b) *Factors Determining the Number.*

A problem of great importance, and one that would seem to lie close to the root of all nuclear phenomena, is that concerning the factors which determine the number of chromosomes. The germ cells of each species have a fixed number of chromosomes, but different species show a very different number, from *Ascaris megalocephala univalens* with 2 up to *Artemia* with about 180. What is it that determines this numerical difference? A consideration of the various thinkable factors allows us to limit the problem somewhat, by excluding certain ones which are not real factors. Here we may consider in what relation to chromosomal number stand centrosomes and achromatic spindle elements, number of nucleoli, mass of nucleus and cell body, form of cell, volume and form of chromosomes.

The size, number and specific peculiarities of the centrosomes seem to have no connection with the number of the chromosomes. The definite number of chromosomes appears in the prophases of mitosis while the nuclear membrane is still intact, and when the centrosomes are only commencing to exert an influence upon the other cell constituents. Even the longitudinal splitting of the chromosomes would appear to be an automatic movement on their part. The centrosomes may well be centres of movements which produce the separation of the daughter chromosomes, but there appears to be no correlation between the centrosomes and the chromosomal number. And this seems also to be the case with regard to the spindle fibres. Central spindle fibres and polar radiations may vary in their phenomena in different generations of the same species, but the number of the chromosomes remains constant in all generations of the germ cells, for the apparent

halving of the number just before the maturation mitoses is not a real halving of the normal number, but only a grouping into pairs. And the mantle fibres, those which connect the centrosomes with the chromosomes, seem in fact to have their number determined to some extent by the number of the chromosomes, and not to determine that number, for the definite number of chromosomes appear in mitosis before the mantle fibres arise. The number of the connective fibres, those which connect corresponding daughter chromosomes in the anaphase, is certainly determined by the number of the chromosomes, for these fibres are stretched-out portions of the linin matrices of the chromosomes. Thus the centrosomes and the achromatic spindle structures may play a part in the distribution of the chromosomes, but apparently have no part in the determination of their number.

As for the true nucleoli, their number, volume and position seem to be in no way regulative of the chromosomal number. The nucleolar number in one species is generally variable, and correlatively also the volume and position, while the chromosomal number is constant; in the paternal germ cells the number of nucleoli is frequently different (and generally smaller) than that in the maternal cells of a species, but in both kind of cells the number of chromosomes is the same.

The mass of the nucleus or of the cell body, or the relative mass of the two, might seem *à priori* to stand in connection with the chromosomal number, yet an examination of the facts shows this is probably not the case. For instance, in one species the huge ovocytes and the much smaller spermatocytes have the same number of chromosomes, and a small ovogonium has the same number of chromosomes as a large ovocyte. And in the case of the ovocyte the volume of the nucleus is relatively small, in the spermatocyte relatively large in proportion to the mass of the cell body, yet the number of the chromosomes is the same. The mass of the chromatin substance may be more or less proportionate to the volume of the nucleus, but the number of the chromosomes appears not to be; the large number of chromosomes in *Artemia* (as determined by Brauer) is not correlated with a large nucleus; and as the figures of the present paper show, cells of approximately the same size from different species may show very different chromosomal numbers. The form of the cell is regulated to great extent by external influences, and variations in the form produce no modification of the chromosomal number.

The volume of the chromosomes in mitosis is dependent upon their number, since the volume of the chromatin stands in more or less direct ratio to the volume of the nucleus. The form of the chromosomes is more or less dependent upon their number, inasmuch as long ribbon-shaped chromosomes occur only where there are a small number, and rounded ones where there are a larger number present. Yet in all the *Hemiptera*, with their considerable differences in chromosomal number, the form of the chromosomes

remains quite constant, each univalent chromosome being generally slightly elongate. Thus the form is not wholly dependent upon the number, but particular groups of *Metazoa* appear characterized by particular forms of chromosomes, as *e. g.* the *Mollusca* with their rod-shaped chromosomes. Then the form of bivalent chromosomes is dependent upon the mode of junction of the component univalent elements. In no sense, however, can the form of the chromosomes be said to determine their number.

So far our considerations lead to only negative results; centrosomes and achromatic spindle structures, nucleoli, absolute and relative mass of nucleus and cytoplasm, cell form and chromosomal form seem not to be factors determining the number of chromosomes. As I attempted to show in a preceding paper on *Peripatus* (1901), the chromosomes must be regarded as individuals of a lower grade than what I termed the "nuclear element," namely, the linin spirem with the chromosomes arranged upon it. The problem is really, then, why does this nuclear element show in one species a certain number of chromatin segregations, in another species a different number? The more recent chemico-physiological studies would tend to show that the chromosomes are centres of metabolic activity, and accordingly the problem of the factors governing the chromosomal number may be closely connected with the phenomena of metabolism; the number of the chromosomes may be dependent upon the nature of the metabolism, as upon either the chemical nature of the chromosomes themselves or upon that of the cell nutriment. The latter might be experimentally tested by changing the food of a species, and observing whether differences in the number of the chromosomes might thereby be obtained. But to conclude that the number of the chromosomes is dependent upon the nature of the metabolism does not solve the problem but only states it more precisely.

Another question which arises in this connection is whether a small or a large number of chromosomes is to be regarded as the primitive condition. *À priori* it would appear probable that at an early phyletic period the number of chromosomes was not fixed for the species but variable, and that by a process of natural selection the number gradually became fixed. But as a species gradually changes into another form the number of chromosomes may also be changed, as will be shown in the next section, so that we may speak of an evolution of the number of chromosomes. On the principle of the law of greater condensation of organs in progressing evolution, it might be that a large number of chromosomes represents a more primitive condition than a smaller number. Within such a group as the *Hemiptera heteroptera*, for instance, forms like the *Belostomatidæ*, *Reduviidæ*, *Capsidæ* and *Phymatidæ* would be primitive in possessing a larger number of chromosomes, while the *Pentatomidæ*, *Scutellariidæ* and *Lygæidæ* in possessing a smaller number should be regarded as more specialized—more highly developed. From such a standpoint as this, the chromosomal number would be of taxonomic

value—it would be a signboard of degree of specialization within the group. From such a point of view it might even be possible to construct a cellular classification which would have great value in that it would employ truly conservative structures. The centrosomes and central spindles have been considered phyletically in this way by Bütschli, Lauterborn, Heidenhain, Calkins and others, but so far the chromosomes have not been considered from such a standpoint, although they in many respects appear more conservative than centrosomes and achromatic spindle structures.

Accordingly, though the present study has not given a solution to the problem of the factors governing the number of chromosomes, except in showing that it must be sought in the phenomena of metabolism, yet it would show that chromosomal number may be employed as a criterion of relationship if it be used with caution and with due consideration of a broad comparative treatment of other structures. And the reason is because the chromosomes seem to be highly conservative, their number constant for the species, and because in a certain sense they represent the most important vital structures. We should not conclude that all forms with *e. g.* ten chromosomes should be ranked as closely related; all with *e. g.* twenty-four as composing another natural group. But within a certain group which has been defined on a broadly comparative basis—such a group as the *Hemiptera heteroptera*—for instance, the chromosomal number would perhaps be a clue to the relative degree of specialization of the species.

(c) *Significance of the uneven normal number.*

One of the most unexpected results of this investigation was the discovery that in some species there is an uneven number of chromosomes in the spermatogonia, *i. e.*, an uneven normal number, whereas heretofore all observation and assumption has been that the normal number is always an even one. Of the *Hemiptera* studied, four species show an uneven number of chromosomes in the spermatogonia, namely, *Alydus eurinus, Harmostes reflexulus, Protenor belfragei* and *Œdancala dorsalis;* in all of these the number is eleven, and in the synapsis stage one chromosome remains univalent while the ten remaining combine to form five bivalent chromosomes. What is the significance and origin of this uneven number?

Now, as far as our facts go, it seems that the number of the chromosomes is constant for the species, and that the paternal and maternal germ cells of a species have the same number; this appears to be one of the points in the correspondence of the ovogenesis and spermatogenesis first determined by Henking (1890) and O. Hertwig (1890). Whenever there is this correspondence in number and valence, then in fertilization, when the paternal chromosomes are added to the maternal, an even number of chromosomes should result, and if the chromosomes maintain their individuality through the succeed-

ing generations, there should be an even number in the spermatogonia and ovogonia. The uneven number discovered in the four species mentioned may have arisen in one of two ways: through bastardization, or through a mitotic abnormality, each of which possibilities may now be considered.

In the case of bastardization of a germ cell with one chromosomal number by a germ cell from another species with a different number, the uneven number eleven might be secured if a paternal germ cell of a species *A*, with the normal chromosomal number twelve, fertilizes a maternal germ cell of a species *B* with the normal number ten. In species *A* the reduction in number of the chromosomes would give six univalent chromosomes in the spermatid, and in species *B* five univalent chromosomes in the matured ovum; conjugation of the two cells would then result in $6 + 5 = 11$ univalent chromosomes. In this way an uneven chromosomal number may have arisen by the conjugation of the germ cells of species with different numbers of chromosomes. But the objection to this view lies in the fact that hybridization of distinct species is generally infertile, and species which would have different chromosomal numbers would probably be quite distinct.

More probably, then, the uneven number may have originated through abnormalities of mitosis, and there would be many possibilities for such an occurrence. (1) It may have arisen in a spermatogonic mitosis, in a species where the ancestral normal number is twelve, by the chromatin of the spirem segregating abnormally into only eleven chromosomes, so that one of these chromosomes would be virtually bivalent. This would seem to have been the origin of the large odd chromosome *x* of *Protenor belfragei*, which appears bipartite in the spermatogonia and also in the spermatocytes, though in the latter it does not conjugate with any other chromosome. Such a case would be a deficiency in the segregation of the chromatin in the prophases of mitosis. (2) Or the uneven chromosomal number may have arisen by an unequal distribution of the chromosomes in the anaphases of mitosis, so that the daughter cells would not receive equal numbers.

Now, whether the uneven chromosomal number had originated through bastardization, or, what would be more probable, through abnormality in mitosis, it is interesting to determine how this number can perpetuate itself through different generations of the species, for my observations show that in the species where it is found it occurs in all individuals. The following table gives in condensed form the mode of reduction of the chromosomal number and valence in the four species in question, the chromatin nucleoli being omitted for the sake of simplicity:

Species.	Spermatogonium.	First Spermatocyte.	Second Spermatocyte.	Spermatid.
Alydus eurinus.	11 univalent.	5 bivalent. 1 univalent.	5 univalent. 1 semivalent.	5 or 6 semivalent.
Harmostes reflexulus.	11 univalent.	5 bivalent. 1 univalent.	5 univalent. 1 semivalent.	5 or 6 semivalent.
Protenor belfragei.	10 univalent. 1 bivalent.	5 bivalent. 1 bivalent.	5 univalent. 1 univalent.	5 semivalent. 1 or 0 univalent.
Œdaneala dorsalis.	11 univalent.	5 bivalent. 1 univalent.	5 univalent. 1 semivalent.	5 semivalent. 1 (or 0 ?) semivalent.

It may appear strange that the chromosomes of the spermatids are classed as semivalent since they are generally considered univalent; as I have explained in an earlier portion of this paper, however, they must be regarded as semivalent on account of the absence of a rest stage between the maturation mitoses, though they are virtually univalent on account of their increase in mass during the growth period. *Protenor belfragei* differs from the three other species in showing a bivalent chromosome in the spermatogonium, which chromosome is consequently bivalent in the first spermatocyte even though it unites with no other during the synapsis stage. All four species have in common the phenomenon that the odd chromosome does not conjugate with any other during the synapsis stage, but remains separate. In *Alydus eurinus*, *Harmostes reflexulus*, and *Protenor belfragei* this odd chromosome does not divide in the second maturation mitosis, but passes undivided into one of the two spermatids. In *Œdancala dorsalis* I was unable to determine its behavior in this mitosis, though I have no reason to suppose that here it behaves differently from the other species. This unequal distribution of the odd chromosome in the second maturation mitosis is evidently in some way dependent upon its not having united with a fellow-chromosome during the preceding synapsis stage. What concerns us particularly at present is the fact that in these species with an uneven normal number of chromosomes, unlike those with an even number, one chromosome (the odd one) is not divided in the second maturation mitosis, but passes undivided into one of the daughter cells (spermatids); half of the spermatids then have six chromosomes and half have only five.

Bearing this point in mind, let us see how the uneven chromosomal number may be perpetuated from individual to individual. This may be occasioned by one of two possibilities. (1) The paternal germ cells having eleven chromosomes in the spermatogonia and either five or six in the spermatids, there is the probability that the maternal germ cells (ova) may have a corresponding number of chromosomes. If a spermatozoon with

five (or six) chromosomes conjugates with an ovum with five (or six), so that each of the conjoints has the same number, an even number would result in the fertilized ovum. But if a spermatozoon with five (or six) chromosomes unite with an ovum with six (or five), the conjoints having then different numbers of chromosomes, the fertilized ovum would have the uneven number eleven; the uneven number would then be perpetuated from individual to individual, so long as the conjugating cells have different numbers of chromosomes, and so long as the odd chromosome does not divide in one of the maturation mitoses. (2) Or germ cells from individuals with an uneven normal number of chromosomes, by conjugating with germ cells from individuals with an even number, would occasion an uneven number in the fertilized ovum. Either of these possibilities would suffice to explain the transference of the uneven number from individual to individual, though the first possibility would appear the more probable.

So far we have considered the origin of the uneven chromosomal number and the mode by which it is perpetuated from individual to individual. We have now to discuss its significance. Most of the *Hemiptera* examined by me show an even normal number of chromosomes; only four showed an uneven number, and in no other *Metazoa* has an uneven number, to my knowledge, been found. The uneven number would accordingly appear to be unusual. It seems to me probable that the uneven number represents a transition stage between a higher number and a lower, or the converse, and it is unusual because the transition stage is probably shorter than the earlier and the later stages. The number of the chromosomes varies quite considerably in the different species of the *Hemiptera heteroptera*, but we cannot suppose that the number was constant for each species from the beginning any more than we can consider that the species have always remained unchanged; there must have been an evolution of the chromosomal number, as there has been of the species. It is quite possible that an even number of chromosomes, as *e. g.* twelve, may have changed into an even number (ten) without first passing through the stage of the uneven number (eleven). This might take place by the number ten appearing simultaneously in both paternal and maternal germ cells through some abnormality or deficiency in mitosis. But it is far more probable that such a mitotic abnormality would not occur coincidently in both kinds of cells—more probable, *e. g.*, that a paternal germ cell, acquiring an abnormal number of chromosomes by some fault in the process of mitosis, would conjugate with a maternal germ cell with the normal number; the result of such a union would be of course an uneven number. On this argument, when the chromosomal number changes, the period of change would be characterized by an uneven number of chromosomes. Ultimately an even number of next lower or next higher order would be reached, and that number must persist longer than the uneven number in view of the fact that uneven numbers are comparative rarities. If both paternal and maternal

germ cells gradually acquired the same uneven number of chromosomes, then by conjugation of such cells, similar numbers of chromosomes being added together, a new even number would result. But there is still another possibility by which the uneven number could pass into an even one. The odd chromosome, at least in the cases here described, does not divide in the second maturation division, and so behaves abnormally. Now such an abnormally behaving chromosome might in time become differentiated from the other chromosomes, and I venture the view that such odd chromosomes are on the way to become chromatin nucleoli. The main fact on which this conclusion is based is that in *Protenor belfragei* it is the odd, the eleventh chromosome—the "chromosome *x*"—which in the spermatocytic growth period evinces the phenomena of a chromatin nucleolus. Then another correspondence is that the chromatin nucleoli in most *Hemiptera* act like the odd true chromosome in usually not dividing in the second maturation division. Here we have an explanation for the origin of those peculiarly modified chromosomes, the chromatin nucleoli, thoroughly in accord with the facts I have described for them: the chromatin nucleoli are modified chromosomes, in point of origin the odd chromosomes which appear in the period of transition from a higher (or a lower) to a lower (or higher) even normal chromosomal number. And there are generally two chromatin nucleoli in the spermatogonia, because the odd chromosome in those cases where there is an uneven normal number had probably been formed in most cases, as it certainly appears to have originated in *Protenor belfragei*, as a union of two univalent chromosomes which had failed to separate from one another in the spirem stage of the spermatogonic mitosis. This also explains why the two chromatin nucleoli are generally placed close together in the monaster stage of the spermatogonium, they having been originally contiguous in the spirem thread.

Such an explanation of the origin of the chromatin nucleoli from the odd chromosomes seems to be in accord with all the facts, and so far may be considered a true explanation. The chromatin nucleoli are modified chromosomes, and it is the odd chromosomes which become thus modified. Conversely, we should expect that chromatin nucleoli would be formed whenever the chromosomal number in changing from a higher to a lower one, or the converse, passes through a transition period of an uneven number. Now, as has been shown in the descriptive part of this paper and tabulated on page 207, all the *Hemiptera* examined have two chromatin nucleoli, but some have a larger number. Wherever there is a larger number we find generally that they are of different volumes, and the question arises: why this difference in volume? The explanation might be that the largest ones are those most recently formed; the smallest those which had been evolved at earlier periods, and which are smaller because they are perhaps diminishing through a gradual degeneration. If the chromatin nucleoli when once formed should always preserve their

original size, there should be no gradations in volume—no degeneration on their part—so that in a given species we could determine by their number how many times the chromosomal number had changed. But when new chromatin nucleoli are formed, the older ones would seem to degenerate in the order of their formation. This assumption would explain the occurrence of very minute chromatin nucleoli found in cells of certain *Hemiptera* along with much larger ones; the minute ones would represent chromatin nucleoli formed at earlier periods, now on the way to total degeneration and disappearance. We might explain the general occurrence of one pair in the spermatogonia, or of one bivalent one in the spermatocytes, on the conclusion already reached in an earlier part of this paper, that the chromatin nucleoli are metamorphosed for a special function different from that of the other chromosomes and so necessary for the nuclear activity; and the reason for their degeneration when new ones are formed, in that a single pair would generally appear to be sufficient for this function, so that not more than one pair would remain in functional activity at one time.

Thus we find that the unexpected discovery of an uneven chromosomal number in the spermatogonia opens the way to an explanation of certain phenomena, and suggests others not anticipated. It suggests that there is a gradual evolution in the numbers of chromosomes; that they have not been fixed from the start, but that with the evolution of the species the chromosomal number changes and at each change probably passes through a period with an uneven number. In the *Hemiptera* this would seem to be, in the forms examined, a change from higher to lower numbers, and in such a change the odd chromosome becomes metamorphosed—becomes a metamorphosed chromatin nucleolus. If attention be given to these points in other groups of animals, there can be little doubt that there, too, will be found occasional examples of uneven normal chromosomal numbers, and probably also in some of these cases the production of structures comparable to the chromatin nucleoli of the Insects. There is great need, first of all, however, to determine for the *Hemiptera* whether in such cases there is a close correspondence between the spermatogonesis and ovogenesis—that correspondence I have assumed, since I have not studied the ovogenesis.

4. *Considerations on the Cycle of the Germ Cells.*

Here shall be considered in succession some points of broader interest which have arisen in the course of my studies on spermatogenesis.

(a) *The sequence of the stages of the cycle.*

In the germ cells of the *Metazoa* there may be seen regular cycles of generations following upon one another. In each cycle may be noted a stage of conjugation of ma-

ternal and paternal cells or stage of fertilization; upon this follow a number of ovogonic or spermatogonic generations, the exact number of which has not yet been determined for any metazoon; the last generation of the ovogonia or spermatogonia give rise to ovocytes or spermatocytes of the first order, and these are characterized by the synapsis stage and growth period when the reduction in the number of chromosomes is effected, the synapsis stage being evidently coincident in all forms with the commencement of the growth period; and finally occur two maturation divisions which result in the formation of ovotids or spermatids. The spermatids undergo an elaborate metamorphosis to become spermatozoa; but since such a metamorphosis is not found in the ovotids, we may disregard this stage, which evidently is far less conservative than the others; from the comparative standpoint the metamorphosis of the spermatozoon is of much less morphological significance than the preceding stages of spermatogenesis, and would appear from the recent investigations to be far more variable.

Thus each germinal cycle shows the following well-marked stages: conjugation or fertilization, a stage of a number of ovogonic or spermatogonic generations, the synapsis stage coincident with the growth period, and the stage of the two maturation divisions. Each such cycle is succeeded by a similar one, and so on indefinitely for an indefinite number of cycles. Now it is unthinkable that a cycle should be without a beginning; it must have been gradually evolved, and some particular stage in it must have been the starting point. What was this first stage? An answer is necessary before we can enter into the discussion of the meaning of the synapsis stage.

It appears to me most probable that the stage of conjugation of the germ cells must be considered the starting point. For from the studies of R. Hertwig and Maupas on Infusoria, it appears probable that conjugation or fertilization is essentially a process of rejuvenation: cells may divide and reproduce for a number of generations asexually, but there comes a period when the cellular vitality diminishes, so that no further reproduction is possible except after rejuvenation afforded by conjugation with another cell. When thus rejuvenated by admixture of substances from the other conjoint, the cell starts upon a new period of generation—the period of conjugation thus being the commencement of a cycle. As we shall see, the synapsis stage is really a delayed part of the process of conjugation, and the growth period is induced by the synapsis of the chromosomes. Having determined the starting point of the germinal cycle, we may now consider the meaning of the synapsis stage.

(b) *The phylogeny of chromosomes and the significance of the synapsis stage.*

In the considerations that follow I assume that through the germinal cycle the chromosomes preserve their individuality from generation to generation—*i. e.*, that a particular

chromosome of one generation is represented in a particular one of a preceding, so that chromosomes are not produced *de novo* in each generation. The evidence for this assumption, as regards the *Hemiptera*, has been already stated above (cf. the heading: "The process of spermatogenesis in the Hemiptera"); other evidence was shown in my study on *Peripatus* (1901), and there also the observations of other workers was considered in some detail so it is not necessary at this point to enter into these particulars. Without this assumption, which is an actuality, as I have shown in some cases, it would be very difficult to determine the meaning of the stages of the germinal cycle; while on this assumption much becomes clear, and the phenomena of the synapsis stage alone are strongly corroborative of this assumption.

Now in the cycle of the germ cells there is a chromosomal peculiarity which has been described by other investigators, but its significance has not been understood; I referred to it in my study of *Peripatus* (1901). In the anaphases of the male and female pronuclei, as in the anaphases of the early cleavage cells, it is characteristic that each chromosome becames vesicular so that at this stage each daughter nucleus appears composed of as many such vesicles as there are chromosomes. Each vesicle has its own limiting wall, and not infrequently the different vesicles may be only loosely connected together; ultimately, however, when the complete rest stage is attained, the boundaries between the vesicles disappear so that the nucleus appears a whole without separated parts.*

Rückert (1895) supposes the chromosomal vesicles to represent a shortened anaphase, occasioned by the rapid sequence of the mitoses in the blastomeres; that this is hardly a correct explanation is seen from the following considerations. From the list of cases just mentioned in the footnote it will be seen that anaphases with vesicular chromosomes are found in the pronuclei and in the earlier cleavage cells—*i. e.*, in nuclei at the beginning of the germinal cycle. I have never seen such vesicular stages in the last generations of ovogonia and spermatogonia, nor to my knowledge has any one else; but in these later

* This vesicular stage of the chromosomes in the anaphases of mitosis has been described by the following workers, though this is probably not a complete list: Remak (1855, cited by Henneguy, 1896, blastomeres of *Batrachia*); Oellacher (1872, egg of Trout); Trinchese (1875, cited by Henneguy, 1896, pole cells of Aeolididae); O. Hertwig (1876, *id. citat.*, blastomeres of *Bufo*); Fol (1879, *id. citat.*, *Toxopneustes* egg); Henneguy (1882, 1891, egg of Trout); Bellonci (1884, cited by Henneguy, 1896, blastomeres of *Axolotl*); Schwarz (1888, *id. citat.*, blastomeres of Trout); Van der Stricht (*id. citat.*, larval epidermis of *Salamandra* and *Triton*, megacaryocytes, leucoblasts and erythroblasts of embryonic liver of Mammals); Mead (1895, 1898, *Chætopterus*, female pronucleus and blastomeres up to 16-cell stage); Foot (1894, 1897, female pronucleus of *Allolobophora*); Sobotta (1897, pronuclei and first cleavage of *Amphioxus*); Kostanecki and Wierzejski (1896, male and female pronuclei of *Physa*); v. Klinckowström (1897, male and female pronuclei of *Prosthocerœus*); Rückert (1895, blastomeres of *Cyclops*); O. Schultze (1887, blastomeres of *Axolotl*); Koelliker (1889, blastomeres of *Siredon*); Van Beneden and Neyt (1887, blastomeres of *Ascaris*); Böhm (1888, male and female pronuclei and first cleavage of *Petromyzon*); Wheeler (1897, female pronucleus of *Myzostoma*, occasionally showing widely separated chromosomal vesicles); Coe (1898, female pronucleus and first cleavage of *Cerebratulus*); Boveri (1888, blastomeres of *Ascaris*).

germinal stages, as well as in apparently all adult tissue cells (compare Flemming, 1882, 1890; Zimmermann, 1890; Rabl, 1885; Torok, 1888), there is generally no such vesicular stage during the anaphase—it is then characteristic of embryonal cells, of those at the commencement of the generative cycle. An explanation of a possible reason for the chromosomal vesicles maintaining their independence was given in my paper on *Peripatus* (1901), where I referred it to the breaking of the linin spirem effected by the reduction mitosis, and maintained that no continuous chromatin spirem could be formed—*i. e.*, no close juxtaposition of the chromosomes be effected until the linin spirem had become restored.

Probably the chromosomal individuality is maintained through all the generations of the cycle, but the chromosomes seem to show their independence most markedly in the early stages, where it is strikingly evinced by their vesicular phenomena. Each vesicle appears to be potentially a little nucleus, with its ova wall, its chromatic reticulum and caryolymph, and sometimes with its own nucleoli. This is very suggestive of the possibility that each chromosome may represent, from the phyletic point of view, a nucleus; and a metazoan nucleus would then be a symbiotic union of as many nuclei as there are chromosomes. Such a conclusion might explain why the chromosomes pass through vesicular phases resembling nuclei in the earlier periods of the cycle.

So far we have seen that in the earlier portion of the germinal cycle the chromosomes remain more disconnected from one another than at later periods; in the later periods, those *e. g.* of the last generations of the spermatogonia and ovogonia, they no longer show vesicular, nuclear-like appearances in the anaphases, and appear to be more dependent upon one another—less independent. Now another line of facts may be considered in this regard. Van Beneden (1883, 1887) first showed that in the fertilized egg of *Ascaris* the paternal and maternal chromosomes remain separated from one another, so that in the prophases of the first cleavage mitosis a paternal and a maternal chromatin spirem is formed; thus Van Beneden concluded a maintenance of the individuality of the pronuclei. Then Rückert (1895) found in the cleavage cells of *Cyclops* that the paternal and maternal chromosomes form two separate groups throughout the mitosis, and that even in the rest stage there is a double nucleus, half paternal and half maternal; in the prophases there is a paternal chromatin spirem distinct from the maternal one. Up to about the 32-cell stage Rückert was able to find these double nuclei, but found that in later cleavage stages they gradually decrease in number. But Rückert is probably in error when he concludes that the separation of the paternal and maternal chromosomes is retained even up to the time of the first maturation mitosis (first pole spindle). He bases this conclusion on the discovery that in the equatorial plane of the spindle at this stage the chromosomes are arranged "ausnahmslos" into two groups. Now here the chromo-

somes are bivalent and eleven in number, so that each group cannot have the same number. Thus out of the cases examined by him, in twelve cases the chromosomes were in groups of relatively equal number (relation of six to five); in two cases, one group had four, the other group seven chromosomes; in three cases, they were arranged in groups of three and eight, respectively; and in one case, in groups of two and nine, respectively. Thus, though there may be at the period of the first maturation mitosis an arrangement of the chromosomes into two groups, yet the variable discrepancy in the number of the chromosomes composing the two groups shows that it is impossible that one group has only paternal chromosomes and the others only maternal, for the reason that at the start (in the fertilized egg) paternal and maternal chromosomes are equal in number. Accordingly, Rückert has shown for *Cyclops* that the maternal and paternal chromosomes form separate groups up to about the 32-cell stage, when the separateness of these groups gradually disappears; and his own descriptions and figures would show that at the time of the first maturation mitosis there is no longer a paternal group of chromosomes separate from a maternal group. There could also be mentioned the observations of other authors to the effect that the paternal and maternal chromosomes compose separate groups in the early cleavage cells, as especially the observations of my colleague, Prof. Conklin, on the eggs of *Crepidula.*

Accordingly, we have seen that in the earlier period of the germinal cycle, at the time of fertilization and the immediately following generations, paternal and maternal chromosomes remain separated from one another, and also that the individual chromosomes show a remarkable degree of independence as evinced by their vesicular phenomena in the anaphases. In the later stages of the germinal cycle, on the contrary, paternal and maternal chromosomes appear no longer to be arranged in separate groups, and the chromosomes themselves are no longer vesicular in the anaphases.

Now for the bearing of all this on the question of the significance of the synapsis stage. At the commencement of the germinal cycle, the stage of conjugation of the germ cells, the chromosomes are more distinct from one another than at any later stage; this distinctness gradually disappears as the cycle progresses, and at the time of the synapsis stage the chromosomes actually join together to form half the normal number of (bivalent) chromosomes. What chromosomes are these which unite to form pairs? Does a paternal chromosome unite with a paternal and a maternal with a maternal, or does a paternal chromosome unite with a maternal one? The following considerations show that the latter view is probably the true one.

First of all, in *Ascaris megalocephala univalens* there is the normal number of two chromosomes; as Brauer (1893 b) has demonstrated, one of these is paternal, the other maternal in origin; since these two unite to form one bivalent one in the synapsis stage,

this would be a union of a paternal with a maternal chromosome. Also in the *Hemiptera*, whenever there are in the spermatogonia two chromosomes which are distinguishable from the others by their greater size, as in several species described in this paper—*e. g.*, *Protenor belfragei*—these two especially large ones always unite together in the synapsis to form one bivalent one much larger than the other bivalent ones, and one ot the large ones does not unite with a small one; now it can be shown that one of these large chromosomes is paternal and the other is maternal. For calling the two large chromosomes of the spermatogonia *a* and *b*, respectively, they unite in the synapsis to form the bivalent chromosome *ab;* the first maturation mitosis (here a reduction division) gives *a* to one daughter cell (second spermatocyte) and *b* to the other; the second maturation division of the one of these daughter cells gives to each spermatid $\frac{1}{2}a$, the corresponding division of the other daughter cell $\frac{1}{2}b$ to each spermatid. What we find in each of these spermatids is only *one* especially large chromosome, and not *two*. Accordingly, in order for there to be *two* in the spermatogonia, the egg cell must furnish *one*, and then that *one*, together with the *one* furnished by the spermatozoon in fertilization, would make up the *two*. Then of the two particularly large chromosomes of the spermatogonium, one would be paternal and one maternal, and since these two unite in the synapsis stage, this would be a union of a paternal chromosome with a maternal chromosome. A case where two particularly large chromosomes are distinguishable in the spermatogonia was selected for discussion, because these two, on account of their peculiarity in size, can be recognized through the maturation divisions; but if the conclusion be true that one of the large chromosomes is paternal and one maternal, and that these two join together in the synapsis, then it would be very probable that each of the other bivalent chromosomes of the spermatocytes represents a univalent paternal chromosome united with a univalent maternal one. This case, as the one of *Ascaris megalocephala univalens*, may be considered very positive cases in favor of the union of paternal with maternal chromosomes in the synapsis stage. There is still another point of view which makes this conclusion very probable. As we have seen, whenever there is an even number of chromosomes in the spermatogonia, exactly half that number of bivalent chromosomes are formed in the synapsis; thus in *Euchistus variolarius* there are fourteen univalent chromosomes in the spermatogonia, and seven bivalent ones in the first spermatocytes. Now seven of these chromosomes are paternal and seven maternal, since the spermatids have only seven. The regular formation of seven bivalent chromosomes in the synapsis stage would be only possible if maternal chromosomes united with paternal ones. For if, on the contrary, paternal chromosomes united with paternal and maternal with maternal, then of the seven paternal chromosomes three bivalent ones could be formed, but there would be left an ununited odd one, and similarly of the seven maternal chromosomes there would remain an ununited (univalent) odd one. But since

in the first spermatocytes of *Euchistus* all the chromosomes are united into pairs, so that there are no chromosomes remaining univalent, it follows that in this case it is impossible that only chromosomes of like parentage should unite together.

These considerations render it very probable that in the synapsis stage is effected a union of paternal with maternal chromosomes, so that each bivalent chromosome would consist of one univalent paternal chromosome and one univalent maternal chromosome. This conclusion allows us to consider the synapsis stage in an entirely new light, and gives an important significance to this stage. The synapsis stage then, which is characterized by the union of chromosomes into bivalent pairs, may be considered the stage of the *conjugation of the chromosomes.* When the spermatozoon conjugates with the ovum there is a mixture of cytoplasm with cytoplasm, of karyolymph with karyolymph, possibly also an intermixture of other substances; but there is then no intermixture of chromatin, for the chromosomes then, as we have seen, remain more separated from one another than at any other stage—in fact, the paternal chromosomes seem to show a repulsion for the maternal, inasmuch as they are arranged in two separate groups. But after this beginning stage of the germinal cycle, the repulsion of the paternal for the maternal chromosomes gradually diminishes, is generally no longer recognizable in the last of the spermatogonic and ovogonic divisions, and in the synapsis stage instead of a repulsion we find a positive attraction between the paternal and maternal chromosomes. The reason for the final union of these chromosomes is obvious: it is evidently to produce a rejuvenation of the chromosomes. From this standpoint the conjugation of the chromosomes in the synapsis stage may considered the final step in the process of conjugation of the germ cells. It is a process that effects the rejuvenation of the chromosomes; such rejuvenation could not be produced unless chromosomes of different parentage joined together, and there would be no apparent reason for chromosomes of like parentage to unite. At the same time the so-called "reduction in the number" of the chromosomes is effected, but this is probably not primal but rather a necessary result of the conjugation of the chromosomes. And here the point may be made that really there takes place no reduction in the number of the chromosomes in the germinal cycle, but "reduction in number" is simply a convenient phrase for expressing that in the synapsis the chromosomes unite to form pairs; no chromosomes have been lost, there is in the strict sense no reduction in number.

So we find that the synapsis stage has a very broad and important significance, of all the stages in the germinal cycle second only to the initial stage of conjugation of the germ cells. In the synapsis stage we see the final process in the conjugation of the germ cells, namely, the conjugation of the chromosomes. Now following immediately upon the synapsis stage comes the growth period of the spermatocytes and ovocytes—that period when the germ cells attain volumes greater than at any other period in the germinal cycle.

Very evidently this great increase in volume is effected by that rejuvenescence of the chromosomes secured by their conjugation. For the chromosomes are centres of metabolic activity, by conjugation of paternal with maternal chromosomes in the synapsis stage their metabolic functions are rejuvenated, and this rejuvenation finds its expression in the great changes of the growth period. So this explanation of the synapsis stage would seem to be in accord with all the facts known at present.

It is quite possible that at an earlier period in the phylogeny, the conjugation of the chromosomes may have taken place at the time of the conjugation of the germ cells, and not have been separated from that stage by a number of generations as in the modern *Metazoa*. But the determination of the original time of occurrence of the conjugation of the chromosomes is highly speculative, and so will not be entered upon here.

It is generally stated (*e.g.* Von Rath, 1893; Rückert, 1894) that the bivalent chromosomes of the spermatocytes and ovocytes of the first order are produced "by the spirem segmenting into only half the normal number of chromosomes." This is not a correct statement, since in the prophases of the first maturation mitosis there is, as I have shown in my paper on *Peripatus*, no stage of a continuous chromatin spirem. Further, this general statement is not at all explanatory of the formation of bivalent chromosomes, for it does not express any reason why the chromosomes should be joined into pairs.

It is to Moore (1895) that we owe the first clear characterization and estimation of the synapsis stage; he divided the germinal cycle into the "first period" (conjugation of germ cells, spermatogonic and ovogonic divisions), the "synaptic phases" (coincident with the growth period), and the "second period" (maturation divisions). It will be seen that my own classification of the stages is somewhat more detailed than Moore's, though it is not necessarily any better. The important characteristic of the synapsis stage is, of course, the union of chromosomes into bivalent pairs; the exact details of this process, which appear to differ in different groups, are of secondary significance.

(c) *The significance of the maturation divisions.*

The two maturation divisions of the *Metazoa* represent the terminal stages of the germinal cycle.

In the Copepods (Häcker, 1895; Rückert, 1894), the Isopods (*Oniscus*, in a just finished paper by my student, Miss Louise Nichols), in the Insects (Von Rath, 1892; Henking, 1890; Montgomery, Paulmier, 1899; McClung, 1900), and in *Peripatus* (Montgomery, 1901) there are well demonstrated cases that one of the maturation divisions is a reduction division (*pseudoreduction*, Rückert, 1894) in that it accomplishes a separation of entire univalent chromosomes from one another. Such a reduction division, a transverse splitting of the chromosomes, is not known for any other generation of the germinal cycle, nor for any somatic generation.

But in *Ascaris* (Brauer, 1893 b), in *Salamandra* (Meves, 1896), in the Rat (Lenhossek, 1898), in *Selachii* (Moore, 1895), and in *Amphiuma* (McGregor, 1899) the authorities cited agree that both maturation divisions are equational. Now it does not seem *à priori* probable that in some *Metazoa* a reduction division should occur, and in others not. The case of *Ascaris* would seem to show no sign of a reduction division, for Brauer's careful study apparently shows that each bivalent chromosome becomes split longitudinally twice; yet Sabaschnikoff's more recent study (1898) would show that another interpretation of the phenomena is possible (but not proved), namely, that the chromatin microsomes may become rearranged into fours in such a way that one of the maturation divisions may be reductional. In the Salamander, Flemming (1887) showed that the mitosis of the first generation of spermatocytes has remarkable peculiarities, so that he named it a "heterotypic" mitosis. The most remarkable of its characteristics is that the chromosomes are longitudinally split in shape like horseshoes, and that they open up into the forms of rings, the ends of the daughter horseshoes retaining their mutual connection. Such a heterotypic mitosis was corroborated by Meves in his description of the spermatogenesis of *Salamandra;* and it has been shown to be characteristic of the first maturation division in *Selachii*, the Rat, and *Amphiuma* by Moore, Lenhossek and McGregor, respectively.* All these writers show that the heterotypic mitosis results in a longitudinal division of the chromosome, and there can be no reasonable doubt of the correctness of their descriptions. But I think they are mistaken in concluding that because the heterotypic division is a longitudinal division of the chromosomes, that therefore it is an equation division. For the chromosomes of these spermatocytes are bivalent—there are just half as many in the spermatocytes as in the spermatogonia. Since there is no loss of chromosomes in the spermatocytes, there must take place a union of univalent chromosomes into pairs during some part of the growth period—*i. e.*, in the synapsis stage. I venture the view that in the Vertebrates either (1) the bivalent chromosomes are formed by every two univalent chromosomes becoming apposed to one another side to side—*i. e.*, along their whole length, so that the two would compose a double horseshoe—or (2) by the two ends of one univalent chromosome becoming closely connected with the two ends of the other, so that the whole would have the form of a ring. From what has been described for these bivalent chromosomes, we know that the longitudinal split does not divide their ends, but the ends are unsplit. Accordingly, it would appear probable that the essential process in the formation of these bivalent chromosomes is that the two ends of one univalent chromosome become united with the two ends of another, while it would be of secondary importance whether the two chromosomes might be apposed along their whole lengths or not.

*Moore states that the second maturation division is also heterotypic, but his figures do not prove his point, which needs reëxamination.

But from this it would follow that the heterotypic mitosis of the first spermatocytes of the Vertebrates is really a reduction division, and results in the separation of whole univalent chromosomes. Then the longitudinal split of such bivalent chromosomes would be really the space between two univalent chromosomes. Thus, though these chromosomes may appear in the prophases longitudinally split, yet a separation of the daughter chromosomes along the line of this split would not be an equational division. The workers on vertebrate spermatogenesis have indeed shown that the bivalent chromosomes are split longitudinally, but since none of them have succeeded in demonstrating how the bivalent chromosomes are formed in the synapsis stage they could not show the significance of this longitudinal split. For *Peripatus* and the *Hemiptera* I have shown that a bivalent chromosome is produced by one end of one univalent chromosome uniting with one end of another; while in the *Vertebrata*, if my interpretation is correct, a bivalent chromosome would be produced by the union of both ends of one univalent chromosome with both ends of another—the spermatogonic chromosome is U-shaped, the spermatocytic chromosome is ring-shaped since it represents two such U-shaped elements with their ends connected. Also in *Peripatus* and the *Hemiptera* there are occasionally ring-shaped chromosomes similar to the heterotypic chromosomes of Vertebrates, and they are formed by the two ends of one univalent chromosome being joined with the two ends of another, instead of one end being joined simply with one end.

This interpretation explains, and the process has never been satisfactorily explained before, why one of the maturation mitoses in Vertebrates is heterotypic: it is a reduction division separating entire univalent chromosomes, and it differs from all other mitoses of the germinal cycle because it is the only one of them which does separate entire chromosomes. If this view is true, then probably all *Metazoa* would have in common the occurrence of one reduction division, and we should no longer be confronted by the discrepancy between *Metazoa* with and those without a reduction division. The occurrence of a reduction division is actually proved for the *Copepoda*, *Insecta*, *Oniscus* and *Peripatus* (I shall not mention other objects where it has been rendered probable but not thoroughly proven); *à priori* we should expect that one would occur in the Vertebrates also, and in the Vertebrates there does occur a peculiar heterotypic division which, as we have seen, can be satisfactorily explained as a reduction division. Accordingly, the term "heterotypic mitosis" might be applied to any mitosis which results in the separation of whole univalent chromosomes, irrespective whether it divides the bivalent chromosome transversely or longitudinally; the term "heterotypic" is indeed most excellent in that it expresses a mitosis "of a type of a different kind," one differing from all other mitoses of the germinal cycle. Of very secondary importance, then, would be the form of the chromosomes—not the form but the way in which the chromosomes divide should be

taken as the criterion of the heterotypic mitosis. This signification would be different from that originally defined by Flemming (1887), but it would certainly be a step toward greater clearness to use "heterotypic" division in the place of the promiscuously used "reduction" division.

Now that we have seen that a reduction division occurs in the *Copepoda*, *Insecta*, *Oniscus* and *Peripatus*, and that the heterotypic division of the *Vertebrata* may be interpreted as a reduction division also, we have to try to explain why such a reduction division occurs. In the synapsis stage there is a conjugation of paternal with maternal chromosomes for the purpose of rejuvenation of the chromosomes as metabolic centres, and this rejuvenation is exemplified in the great metabolic activity of the growth period. Now, R. Hertwig and Maupas have shown for the *Infusoria* that the two conjoints remain for only a certain period in apposition, and that when the interchange of nuclei necessary for rejuvenation has been accomplished the conjoints separate. Of course it is not a true analogy to compare conjugating *Infusoria* (*i. e.*, whole cells) with conjugating chromosomes (*i. e.*, portions of cells). But still it is very probable that two chromosomes unite temporarily for the same reason that two *Infusoria* do, that is, for an interchange of substances; and when the chromosomes have accomplished this interchange there would no longer be any necessity for continued apposition, so they tend to separate from one another. It is the reduction division in *Metazoa* which accomplishes the complete separation, though it may commence in the prophases of this division. It is conceivable that the conjugated chromosomes might separate as they had come together, without the intervention of a mitosis. But in the *Metazoa*, so far as we know, they become separated only by the agency of a mitosis, and that is the reduction mitosis. At the beginning of the germinal cycle there is a repulsion of paternal and maternal chromosomes for one another, during the synapsis a strong attraction, and at the end of the germinal cycle a repulsion again, but not a repulsion so strong as to distribute the chromosomes into two groups in the spermatid and ovotid. Only by a reduction division can paternal and maternal chromosomes become wholly separated, for only then do the interchromosomal linin fibres (persisting portions of the linin spirem: compare my paper on *Peripatus*) become broken.

The question is complicated because another maturation division occurs, an equational division: in the *Insecta*, *Oniscus*, *Peripatus* and the Vertebrates the reduction division precedes the equational, in the *Copepoda* (according to Rückert, 1894) the reverse is the case. It is not difficult to explain why an equation division should occur at this time, for the cell has increased in volume very greatly during the growth period, and great increase in volume (increase beyond the individual mass) would appear to be a main factor in inducing cell division. With this increase in volume of the cell the chromosomes also increase in volume (though by no means in a direct ratio), and each univalent

component of a bivalent chromosome divides into two longitudinally (equationally), this being the usual mode of division of a chromosome—in fact the only known method of reproduction of univalent chromosomes. From this standpoint the growth period would be the inducer of the equational maturation mitosis, and this mitosis would be strictly comparable physiologically to any other equation mitosis of the germinal cycle. But at the time that the cell is preparing for this equation division, paternal and maternal chromosomes, having accomplished the purpose which occasioned their conjugation, show a tendency to repulsion for each other, and so evince the need of becoming disconnected. The cell already started into mitotic activity would offer mechanical possibilities to effect the separation of the maternal from the paternal chromosomes, so that instead of a single mitotic process there are two in rapid succession, sometimes with not a trace of a rest stage in between, one separating entire univalent chromosomes, the other separating the halves of each univalent chromosome. It would be very enticing to enter here into the mechanics of this mitosis, which would be practically a determination of the points of apposition of the mantle fibres on the chromosomes; but such an inquiry is hardly germane to the present discussion. The point to be made is that in the *Metazoa* there follows after the growth period an equation mitosis, because in the growth period the cell has increased beyond the normal size; and that a reduction division occurs about the same time in order to affect a complete separation from one another of the paternal and maternal chromosomes, which, having accomplished the purpose for which they conjugated, show again a repulsion for each other. The growth period is the inducer of the equation division, the mutual repulsion of chromosomes of different parentage the inducer of the reduction division. The chromosomes in the late anaphases, after the maturation divisions, become vesicular and so show a great degree of mutual independence, because the reduction division had severed their linin connections.

In conclusion, it would be very interesting to enter into the question of the parallelism of the germinal cycle in *Metazoa* and *Protozoa*, as has been done by Henking and Moore (1895). However, it would be well first to have ascertained the significance in the cycle of the *Metazoa*, as far as that can be done without reference to the states in the *Protozoa*. The chromosomes are the cell components on which the problem can be best studied.

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Explanation of the Plates.

All figures have been drawn with the camera lucida at the level of the base of the microscope, with the homogeneous immersion objective $\frac{1}{12}$ of Zeiss and ocular 4, tube length 180 mm. In the majority only the chromatin nucleoli, chromosomes and true nucleoli together with the outline of the nucleus or cell body has been drawn ; and in the majority of figures representing lateral views of monaster stages, the mantle fibres, connective fibres, and centrosomes are the only structures shown beside the chromatin elements. For *Euchistus variolarius*, however, the various structures are represented in detail, and this is also the case with some of the figures of various other species.

The following abbreviations have been employed (for others not here mentioned the descriptive text must be referred to):

C. Mb., cell membrane.
N., true nucleolus (plasmosome).
N. 2, chromatin nucleolus (modified chromosome).
N. Mb., nuclear membrane.

Plate 1.

Euchistus variolarius, Figs. 1–19

Fig. 1. Nucleus of spermatogonium, rest stage.
Figs. 2, 3. Pole views of spermatogonic monasters.

Figs. 4, 5. Lateral views of synapsis stages, only a few of the chromatin elements shown.
Fig. 6. Nucleus, synapsis, showing two whole bivalent chromosomes.
Fig. 7. One whole bivalent chromosome, end of the synapsis stage.
Figs. 8, 9. Postsynapsis stages, in each three whole bivalent chromosomes shown.
Fig. 10. Telophase of the spermatocyte, showing two whole bivalent chromosomes.
Fig. 11. Late telophase, showing eight bivalent chromosomes.
Fig. 12. Rest stage of spermatocyte.
Figs. 13, 14. Early prophases of first maturation division, each figure showing two whole bivalent chromosomes.
Fig. 15. Two whole bivalent chromosomes, a little later stage than the preceding, showing their linin connections.
Figs. 16, 17. Later prophases, only in Fig. 16 are all bivalent chromatin elements shown; in Fig. 17 the centrosome pairs on the surface of the nucleus.
Fig. 18. Lateral view of the monaster of the first maturation mitosis, all chromatin elements shown.
Fig. 19. Pole view of the same stage.

Euchistus tristigmus, Figs. 20–26.

Fig. 20. Pole view of spermatogonic monaster.
Figs. 21, 22. Nuclei of first spermatocytes, rest stage.
Figs. 23, 24. Pole views of monaster, first maturation division.
Fig. 25. Lateral view of spindle, first maturation division (showing all chromatin elements).
Fig. 26. Second spermatocyte, chromatin elements not definitely arranged in the equator of the spindle.

Podisus spinosus, Figs. 27–29.

Fig. 27. Pole view of spermatogonic monaster.
Fig. 28. Telophase of first spermatocyte, nucleus.
Fig. 29. Pole view of monaster, first maturation mitosis.

Mormidea lugens, Figs. 30–33.

Fig. 30. Nucleus of spermatogonium, commencement of prophase.
Fig. 31. Pole view of spermatogonic monaster.
Fig. 32. Nucleus, postsynapsis stage.
Fig. 33. Pole view of monaster, first maturation mitosis.

Peribalus limbolaris, Figs. 34–37.

Fig. 34. Spermatogonic nucleus, rest stage.
Fig. 35. Pole view of spermatogonic monaster.
Fig. 36. Nucleus of first spermatocyte, rest stage.
Fig. 37. Pole view of monaster, first maturation mitosis.

Cosmopepla carnifex, Figs. 38–41.

Fig. 38. Pole view of spermatogonic monaster.
Fig. 39. Nucleus of first spermatocyte, rest stage.
Fig. 40. Lateral view of the spindle, first maturation mitosis, showing all chromatin elements.
Fig. 41. Pole view of the same stage.

Nezara hilaris, Figs. 42–45.

Figs. 42, 43. Spermatogonic nuclei, commencement of prophase.
Fig. 44. Pole view of spermatogonic monaster.
Fig. 45. Nucleus of first spermatocyte, early telaphase.

Brochymena sp., Figs. 46–49.

Fig. 46. Spermatogonic nucleus, rest stage.
Fig. 47. Pole view of spermatogonic monaster.

Plate II.

Brochymena sp., Figs. 48, 49.

Fig. 48. Nucleus of first spermatocyte, telaphase.
Fig. 49. Pole view of monaster, first maturation mitosis.

Perillus confluens, Figs. 50–53.

Fig. 50. Spermatogonic nucleus, rest stage.
Fig 51. Spermatogonic monaster, pole view.
Fig. 52. Nucleus of first spermatocyte, rest stage.
Fig. 53. Pole view of monaster, first maturation mitosis.

Cænus delius, Figs. 54–63.

Fig. 54 Spermatogonic nucleus, rest stage.
Fig. 55. Spermatogonic monaster, pole view.
Figs. 57, 58. Nuclei of first spermatocytes, rest stage.
Figs. 59, 60. Pole views of monasters, first maturation mitosis.
Fig. 61. Lateral view of the same stage, showing all the chromatin elements.
Fig. 62. Pole view of monaster, first maturation mitosis.
Fig. 63. Nucleus of first spermatocyte, rest stage.

Trichopepla semivittata, Figs. 64–69.

Fig. 64. Spermatogonic nucleus, rest stage.
Fig. 65. Spermatogonic monaster, pole view.
Fig. 66. Nucleus of first spermatocyte, synapsis.
Fig. 67. Nucleus of first spermatocyte, rest stage.
Fig. 68. Pole view of monaster, first maturation mitosis.
Fig. 69. Lateral view of slightly earlier stage, showing all the chromatin elements.

Eurygaster alternatus, Figs. 70, 71.

Fig. 70. Nucleus of first spermatocyte, rest stage.
Fig. 71. Pole view of monaster, first maturation mitosis.

Anasa tristis, Figs. 72–76.

Figs. 72, 73. Spermatogonic nuclei, rest stage.
Fig. 74. Spermatogonic monaster, pole view.
Fig. 75. Nucleus of first spermatocyte, telaphase
Fig. 76. Pole view of monaster, first maturation mitosis.

Anasa armigera, Figs. 77, 78.

Fig. 77. Spermatogonic monaster, pole view.
Fig. 78. Pole view of the spindle of the first maturation division, the chromatin elements not yet definitely arranged in the plane of the equator.

Anasa sp., Figs. 79–83.

Fig. 79. Spermatogonic nucleus, rest stage.
Fig. 80. Spermatogonic monaster, pole view.
Fig. 81. Nucleus of first spermatocyte, rest stage.
Fig. 82. Pole view of monaster, first maturation division.
Fig. 83. Lateral view of four bivalent chromosomes, monaster stage of first maturation mitosis, the poles of the spindle outside of the plane of the section.

Metapodius terminalis, Figs. 84–87.

Fig. 84. Spermatogonic nucleus, rest stage.
Fig. 85. Spermatogonic monaster, pole view.
Fig. 86. Nucleus of first spermatocyte, telaphase.
Fig. 87. Pole view of monaster, first maturation mitosis.

Chariesterus antennator, Figs. 88–90.

Fig. 88. Nucleus of first spermatocyte, telaphase.
Fig. 89. Lateral view of monaster stage of the first maturation mitosis, showing all the chromatin elements.
Fig. 90. Pole view of the same stage.

Alydus pilosulus, Figs. 91–95.

Fig. 91. Spermatogonic nucleus, rest stage.
Fig. 92. Spermatogonic monaster, pole view.
Figs. 93, 94. Nuclei of first spermatocytes, rest stage.
Fig. 95. Pole view of monaster, first maturation mitosis.

Plate III.

Alydus eurinus, Figs. 96–102.

Fig. 96. Spermatogonic monaster, pole view.
Fig. 97. Nucleus of first spermatocyte, telaphase.
Fig. 98. Pole view of monaster, first maturation mitosis.
Fig. 99. Lateral view of the same stage.
Fig. 100. Pole view of monaster, second maturation mitosis.
Figs. 101, 102. Spermatids at close of second maturation mitosis.

Corizus lateralis, Figs. 103–106.

Fig. 103. Nucleus of first spermatocyte, telaphase.
Figs. 104, 105. Pole views of monasters, first maturation mitosis.
Fig. 106. Oblique view of the spindle of the first maturation mitosis, before the chromosomes have taken their definite position in the equator.

Harmostes reflexulus, Figs. 107–117.

Fig. 107. Spermatogonic nucleus, rest stage.
Figs. 108–110. Spermatogonic monasters, pole views.
Fig. 111. Nucleus of first spermatocyte, rest stage.
Fig. 112. Pole view of monaster, first maturation mitosis.
Fig. 113. Lateral view of the same stage, showing all the chromatin elements.

Figs. 114, 115. Pole views of monasters, first maturation mitosis.
Fig. 116. Lateral view of the same stage, showing all the chromatin elements.
Fig. 117. Pole view of spermatid at the close of the second maturation division.

Protenor belfragei, Figs. 118–141.

Fig. 118. Spermatogonic nucleus, early prophase.
Figs. 119–123. Spermatogonic monasters, pole views.
Fig. 124. Nucleus of first spermatocyte, synapsis.
Figs. 125–128. Lateral views of the large chromosome *x*, from nuclei in the late synapsis stage.
Figs. 129, 130. Nuclei of first spermatocytes, telaphase.
Figs. 131–134. Nuclei of first spermatocytes in successive prophases.
Figs. 135, 136. Lateral views of successive monaster stages, first maturation mitosis, all the chromatin elements shown in Fig. 135.
Fig. 137. Pole view of the stage of Fig. 136.
Fig. 138. Lateral view of the anaphase, first maturation mitosis.
Fig. 139. Pole view of second spermatocyte, chromosomes not definitely arranged in the equator of the spindle.
Fig. 140. Lateral view of anaphase, second maturation mitosis.
Fig. 141. Still later anaphase.

Plate IV.

Cymus augustatus, Figs. 142–144.

Fig. 142. Lateral view of monaster, first maturation mitosis.
Fig. 143. Pole view of one daughter cell (second spermatocyte) of the dyaster stage of the first maturation mitosis, the univalent chromatin elements seen laterally.
Fig. 144. Pole view of one chromosome plate, metakinesis of first maturation mitosis, chromatin elements seen on end view, except the one marked *a*.

Ichnodemus falicus, Figs. 145–148.

Fig. 145. Spermatogonic monaster, pole view.
Fig. 146. Nucleus of first spermatocyte, late prophase, showing all the chromatin elements.
Figs 147, 148. Pole views of monasters, first maturation mitosis, in Fig. 148 two of the chromosomes viewed laterally.

Peliopelta abbreviata, Figs. 149–151.

Fig. 149. Spermatogonic monaster, pole view.
Fig. 150. Pole view of monaster, first maturation mitosis.
Fig. 151. Lateral view of same stage, two of the large chromosomes not shown.

Œdancala dorsalis, Figs. 152–158.

Fig. 152. Spermatogonic nucleus, rest stage.
Figs. 153, 154. Spermatogonic monasters, pole views.
Fig. 155. Nucleus of first spermatocyte, rest stage.
Fig. 156. Pole view of monaster, first maturation mitosis.
Fig. 157. Lateral view of the same stage, three of the chromosomes not shown.
Fig. 158. Lateral view of the anaphase, first maturation mitosis

Oncopeltus fasciatus, Figs. 159–171.

Fig. 159. Spermatogonic nucleus, early prophase.
Fig. 160. Spermatogonic monaster, pole view.

Figs. 161–165. Nuclei of first spermatocytes, rest stage.
Fig. 166. Nucleus of first spermatocyte, late prophase, showing all the chromatin elements.
Figs. 167, 168. Pole views of monasters, first maturation mitosis.
Fig. 169. Lateral view of same stage, five of the chromosomes not shown.
Fig. 170. Lateral view of anaphase, first maturation mitosis.
Fig. 171. Pole view of second spermatocyte, chromatin elements not definitely arranged in the equator of the spindle.

Leptopterna dolabrata, Figs. 172–176.

Figs. 172–175. Nuclei of first spermatocytes, growth period.
Fig. 176. Pole view of monaster, second (?) maturation mitosis.

Calocoris rapidus, Figs. 177–188.

Fig. 177. Spermatogonic monaster, pole view.
Figs. 178–180. Nuclei of first spermatocytes, telaphase.
Fig. 181. Oblique lateral view of the spindle before the chromosomes are arranged in the plane of the equator, first maturation mitosis.
Figs. 182–184. Oblique lateral views of monasters, first maturation mitosis.
Figs. 185, 186. Pole views of the same stage.
Figs. 187, 188. Pole views of monasters, second maturation mitosis.

Pœcilocapsus lineatus, Figs. 189, 190.

Fig. 189. Nucleus of first spermatocyte, early telaphase.
Fig. 190. Pole view of monaster, first maturation mitosis.

Plate V.

Pœcilocapsus goniphorus, Figs. 191–198.

Figs. 191–195. Nuclei of first spermatocytes, rest stage.
Figs. 196, 197. Pole views of monasters, first maturation mitosis.
Fig. 198. Lateral view of the same stage.

Phymata sp., Figs. 199–203.

Fig. 199. Spermatogonic nucleus, rest stage.
Fig. 200. Spermatogonic monaster, pole view.
Fig. 201. Nucleus of first spermatocyte, telaphase.
Fig. 202. Pole view of monaster, first maturation mitosis.
Fig. 203. Lateral view of same stage.

Coriscus ferus, Figs. 204–206.

Figs. 204, 205. Nuclei of first spermatocytes, telaphase and rest stages respectively.
Fig. 206. Pole view of first spermatocyte, the chromosomes not definitely arranged in the plane of the equator of the spindle.

Acholla multispinosa, Figs. 207–211.

Fig. 207. Spermatogonic monaster, pole view.
Figs. 208, 209. Nuclei of first spermatocytes, early prophase.
Fig. 210. Pole view of monaster, first maturation mitosis.
Fig. 211. Pole view of monaster, second maturation mitosis.

Sinea diadema, Figs. 212–218.

Figs. 212, 213. Nuclei of first spermatocytes, telaphase.

Figs. 214, 215. Pole views of monasters, first maturation mitosis.

Fig. 216. Lateral view of the plurivalent chromosome of the first maturation mitosis, showing the mantle fibre attachments.

Figs. 217, 218. Lateral views of successive anaphases, first maturation mitosis.

Limnobates lineata.

Fig. 219. Nucleus of first spermatocyte, rest stage.

Prionidus cristatus, Figs. 220–225.

Figs. 220-222. Spermatogonic nuclei, rest stage.

Figs. 223, 224. Spermatogonic monasters, pole views.

Fig. 225. Nucleus of first spermatocyte, rest stage.

Milyas cinctus, Figs. 226–228.

Figs. 226–228. Nuclei of first spermatocytes, rest stage.

Hygotrechus sp., Figs. 229–231.

Fig. 229. Spermatogonic monaster, pole view.

Fig. 230. Nucleus of first spermatocyte, synapsis stage.

Fig. 231. Pole view of monaster, first maturation mitosis.

Limnotrechus marginatus, Figs. 232, 233.

Fig. 232. Nucleus of first spermatocyte, rest stage.

Fig. 233. Pole view of monaster, first maturation mitosis.

Pelocoris femorata.

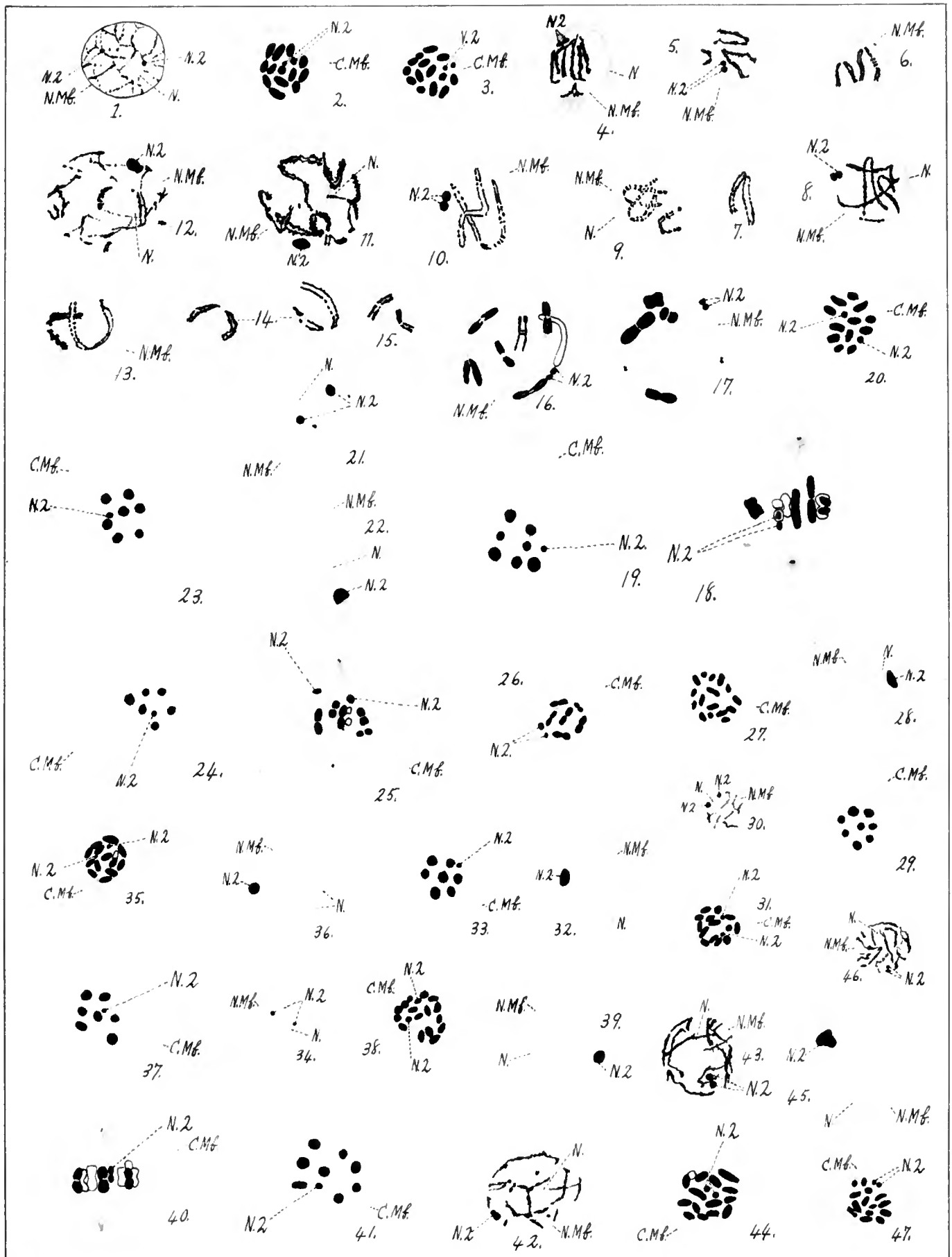
Fig. 234. Spermatogonic monaster, pole view.

Zaitha sp., Figs. 235–238.

Fig. 235. Spermatogonic nucleus, rest stage.

Figs. 236, 237. Spermatogonic monasters, pole views.

Fig. 238. Pole view of monaster, first maturation mitosis.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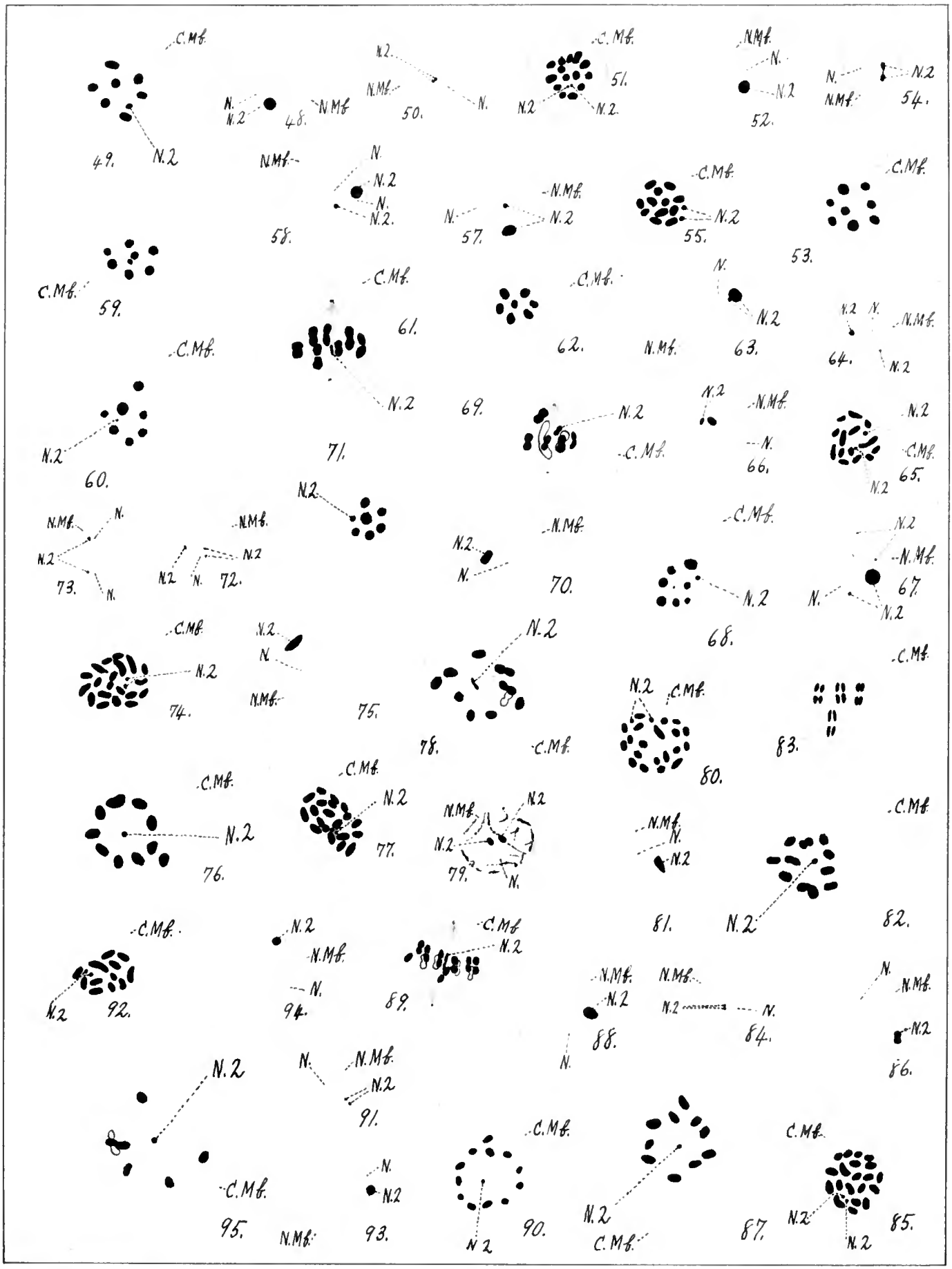
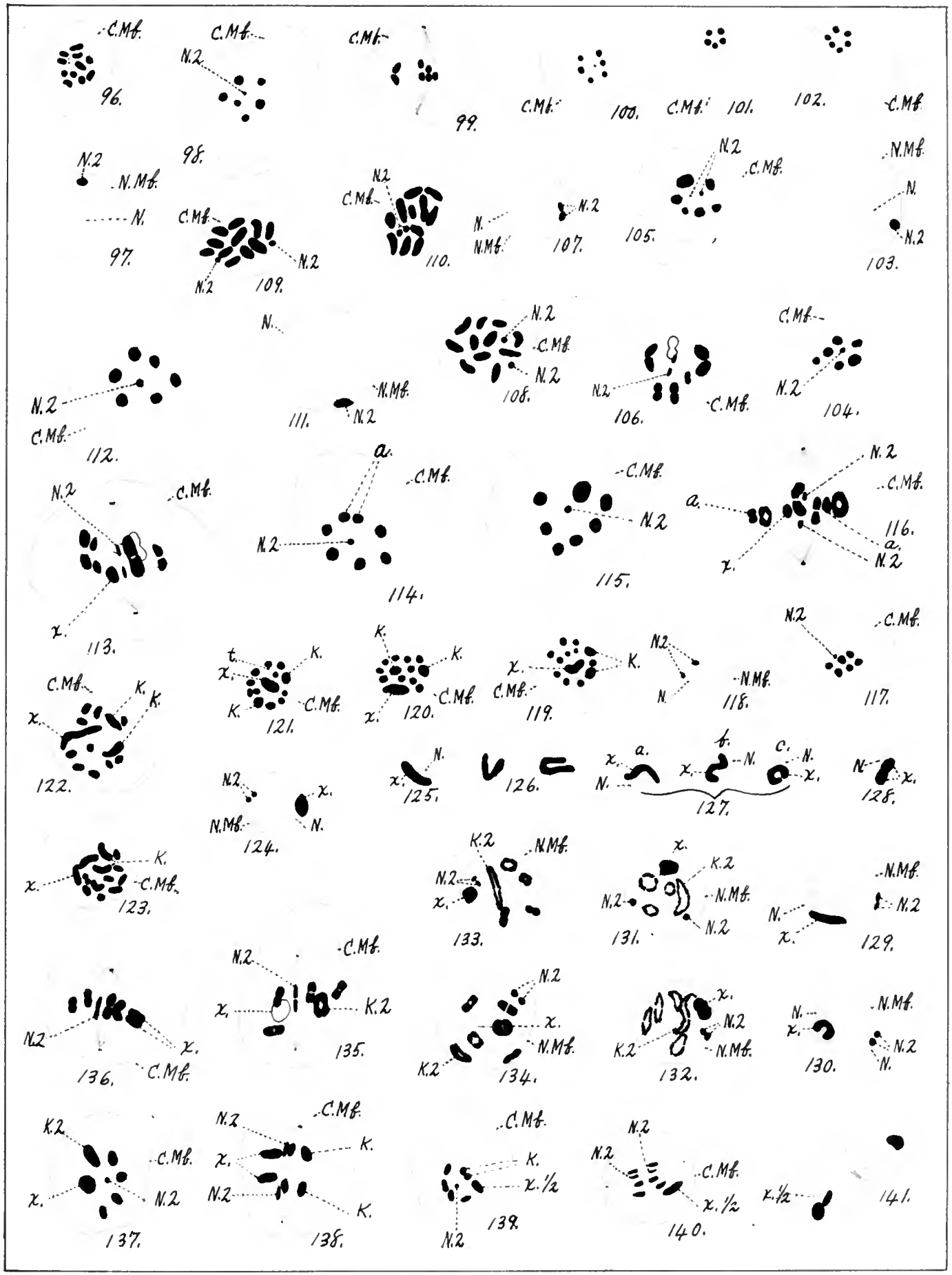
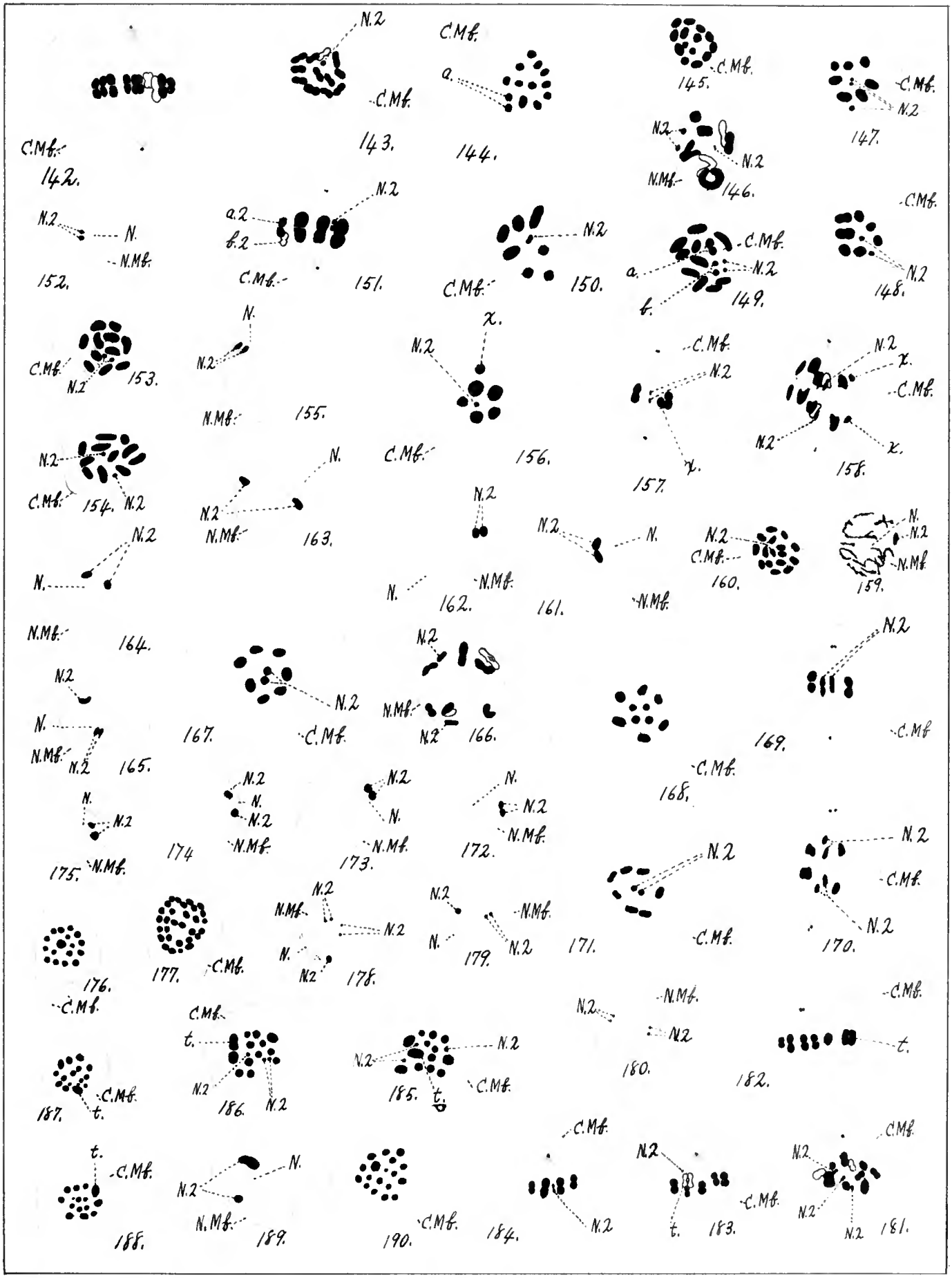


Montgomery, Del. Plate 2.



Montgomery, Del. Plate 3.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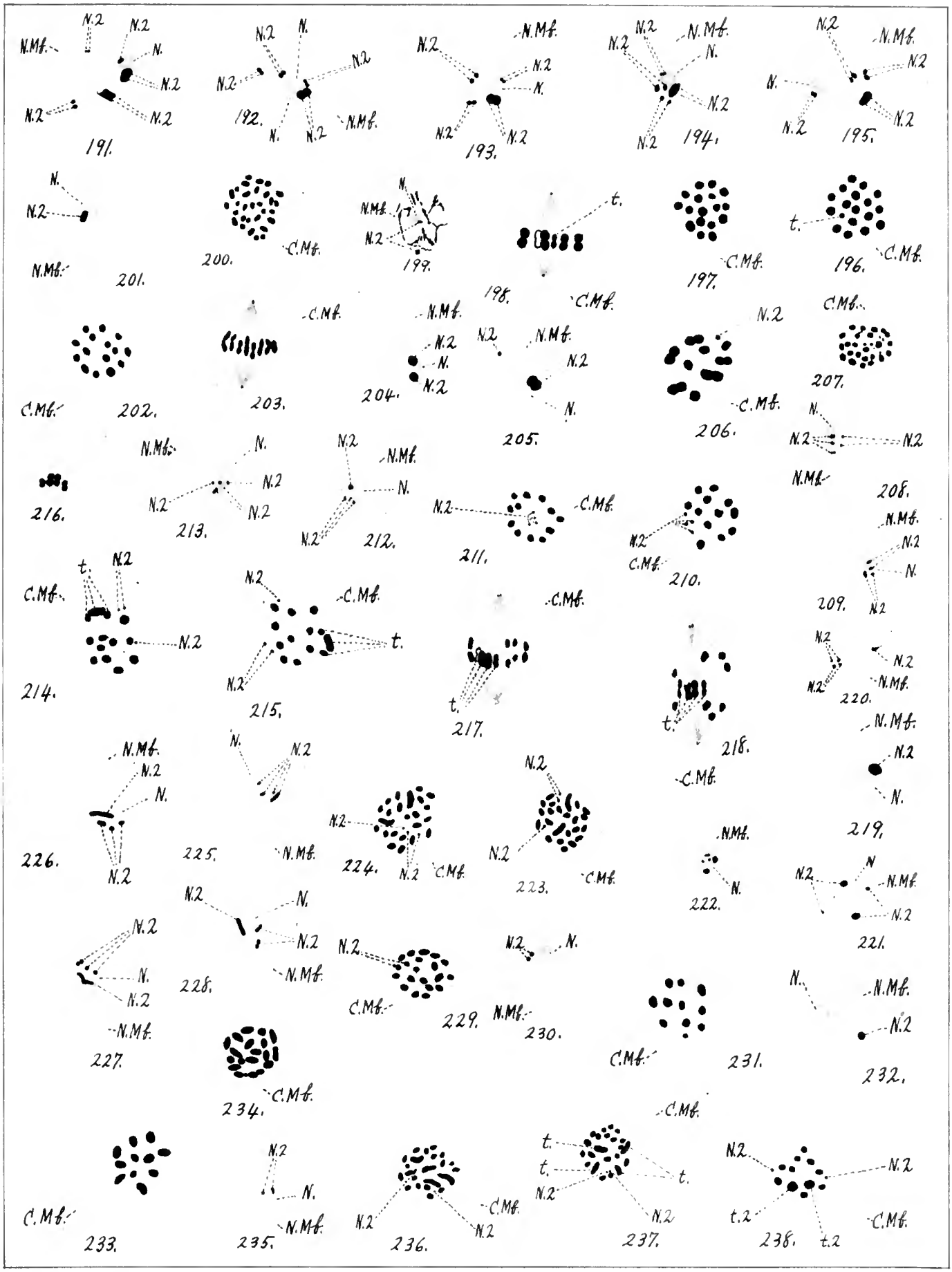
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